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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

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JUNE, 1903, TO NOVEMBER, 1903.

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BIOLOGICAL BULLETIN.

FORM REGULATION IN CERIANTHUS, II.

The Effect of Position, Size and Other Factors upon Regeneration.—(*Continued.*)

C. M. CHILD.

II. Discussion of Results.

The Factor of Position.

In the experiments described above, which were selected from a large number, the effect of position on regeneration has appeared in every case. Considering first the effect of position on the rapidity of regeneration, we find that in each series the rapidity of regeneration is dependent on the position which the piece occupied in the parent body, a decrease in the rapidity of regeneration occurring with increasing distance from the oral end. This relation holds for the aboral ends of pieces as well as for the oral ends, though the regenerative phenomena at the aboral ends are in most cases less sharply defined than at the oral ends. Further analysis is possible in such series as 54 and 55. The oral ends of the two sets of pieces 54*A* and 55*A* are situated at approximately the same level of the body, but the aboral ends of the pieces 54*A* are much nearer the aboral end of the parent body than those of 55*A*. The data given above for this series show very clearly that the rapidity of regeneration at the oral ends of both sets of pieces was equal, except in the final stages, while at the aboral ends it was much greater in the shorter pieces, *i. e.*, those in which the aboral cut surface was near the oral end of the parent body. From these experiments we may conclude that the two ends of a piece regenerate independently of each other, at least to a certain extent, the result depending in each case on the level which a particular cut surface occupied in the parent body.

Near the aboral end the regenerative capacity is reduced nearly

or quite to zero, cut surfaces at this level showing no regeneration beyond the union of ectoderm and entoderm over the cut muscular layer.

The influence of temperature in determining the level at which the regenerative power is reduced to zero will be discussed in another section. As regards the amount of regeneration, the effect of position is similar to its effect on rapidity. Not only is there a decrease in the rapidity of regeneration toward the aboral ends, but the total amount of oral or aboral regeneration also decreases in the same manner. In certain cases, as in Series 54, 55 and 35, the difference in size of pieces may counteract the effect of position in later stages of regeneration, so that short pieces show slightly less oral regeneration than long pieces with oral ends at the same level (Series 54*A* and 55*A*), or the amount of oral regeneration on long aboral pieces may equal that on much shorter pieces from a region nearer the oral end (Series 35, *A* and *B*). But aside from exceptions of this kind the rapidity and the amount of regeneration depend upon the level in the parent body from which the cut surface is taken, both being greatest at the oral end and decreasing aborally.

The existence of a difference in regenerative power at different levels of the body being established, the question as to its nature and significance next requires consideration. I believe that it may be due in part to a difference in the general reactive capacity of the tissues in the different regions, *i. e.*, to a decrease in the reactive power of the tissues with increasing distance from the oral end. That such a difference does exist is indicated by various facts in the normal anatomy and physiology of the animal. We must consider first the anatomical features which bear upon this point. There is a marked decrease in the thickness of the body-wall and especially of the muscular layer, toward the aboral end; the number of mesenteries also decreases toward the aboral end, until only a single pair remains; all new mesenteries appear first at the oral end and extend gradually aborally, thus indicating that growth in circumference begins orally. The physiological differences of different regions which have been noted are: the greater sensitiveness of the oral region to tactile and other stimuli, and the greater contractility of the muscles of this region when stimulated.

Since I am convinced that the power of regeneration is due, not to any special regulatory mechanism, but to those properties of organized matter which cause "normal" or "typical" growth and differentiation, I believe that the differences in regenerative power and the differences in normal anatomical and physiological reactive capacity must all depend, at least in some degree, on the general reactive capacity of the tissues.

The differences in reactive capacity in different regions may be due to differences in the intensity of metabolic change, or they may depend upon other conditions. It is of interest to note in this connection that the differentiation and concentration of nervous tissue is greatest about the oral end in the actinians. It is at least not impossible that this fact is correlated in some manner with those above mentioned. For the present, however, the possibility of such a correlation is merely suggested as the data are still insufficient for positive conclusions.

There is also a possibility that the difference in the rapidity and amount of regeneration at different levels may be due in part to differences in internal pressure resulting from circulatory currents in the enteron. Discussion of this phase of the problem is, however, reserved for a future time.

The Factor of Size.

My observations along this line are to a large extent merely incidental, since, owing to the relatively slight influence of this factor, its effects were not clearly recognized until it was too late to complete the series of experiments necessary for further study. Certain conclusions may, however, be drawn from my work and these are briefly mentioned here.

It is evident from Series 54 and 55 that great differences in size of pieces do not affect the rapidity of regeneration in earlier stages. The pieces 54*A* and 55*A*, although widely different in size, regenerate orally with equal rapidity except in the final stages. Aborally the smaller piece regenerates more rapidly than the larger because its aboral end lies in a region of greater cellular activity. As regards the total amount of regeneration at both ends the small piece exceeds the large piece, because of its position in the parent body. The only effect of size was noted

at the final stage of the experiment when it was seen that the smaller pieces were falling behind the larger pieces with respect to size of the regenerated structures. In Series 35 the possible effect of size upon the final result was noted, but here also there was no visible effect during the earlier stages.

The results afforded by these two series are confirmed by a large number of experiments. In no case, when pieces were above a certain minimal size, could any effect of size upon rapidity of regeneration be observed during the earlier stages. In the later stages it was found that regeneration became less rapid and ceased in the smaller pieces earlier than in the larger. Even in this respect differences were slight in pieces of widely different size.

The amount of regeneration is then not proportional to the size of the piece. As regards both oral and aboral cut surfaces smaller pieces show a relatively much greater amount of regeneration than larger pieces ending at the same level. In a piece one tenth the length of the body the tentacles regenerate at first with the same rapidity as in a piece nine times as long. Only after the tentacles are well formed and several millimeters in length does a difference appear. In many pieces so short that they appeared to consist only of the regenerated disc and tentacles these organs were of the same size up to a late stage as in pieces many times as large.

As regards the nature of the effect of size in regeneration in *Cerianthus* and in many other forms a few points which have suggested themselves to me may be discussed in the hope of aiding in the analysis of this phase of the problem. It may be stated as a general rule that as regeneration advances the stimulus to regeneration diminishes. We may suppose that a certain amount of material or energy is necessary for the production of regenerated structures of a certain size. This energy is derived from the substance of the body in cases like the present where no food is supplied. The smaller pieces possess of course a smaller amount of material or energy available for regeneration. This is sufficient, however, for the earlier stages, but as the amount diminishes it is probably given up less readily by the other tissues. The well-known regulative power of animal tis-

sues during starvation supports this view. Now as regeneration advances it is clear that in the smaller pieces the point where the stimulus to regeneration is no longer sufficient to cause the transfer of material or energy from the other tissues, would be reached earlier than in larger where the total supply of available material is much greater. Consequently regeneration in the smaller piece is retarded earlier than in the larger, even though the stimulus may be as great. The small piece is not necessarily exhausted, for if a new cut is made it may regenerate again, but the reserve supplies are held so closely by the other tissues that the decreasing regenerative stimulus is insufficient to render them available. A new cut surface means an increased stimulus, and under these conditions material which was not available during the later stages of the preceding regeneration may now be made available by the increased stimulus, though in this second regeneration the supply will run out sooner than before.

In the larger pieces of *Cerianthus* it is probable that the stimulus to regeneration ceases, or reaches the level of normal reparative stimuli, before a shortage of material occurs. A convenient designation for the condition of the smaller pieces is "relative exhaustion." They are not absolutely exhausted, since they may regenerate further under sufficiently powerful stimulus; they are, however, exhausted so far as the stimulus to which they are subjected is concerned. There is nothing new in the idea of relative exhaustion; it is a well-known phenomenon in biology and its rôle in regulative phenomena is undoubtedly important.

It has been impossible to determine with any degree of exactness the minimal size of pieces in which regeneration is possible. In small pieces the inrolling of the cut margins becomes so irregular that the piece often takes a form in which normal regeneration is impossible simply because of the relative position of parts. Moreover, since the regenerative power is different at different levels the minimal size of pieces differs according to the region of the body from which they are taken. Short pieces in the œsophageal region are not available for the aboral cut margin of the œsophagus unites with the aboral cut margin of the body-

wall, thus producing a condition in which the œsophagus opens aborally as well as orally to the exterior and the enteron is completely closed. As will be shown at another time, regeneration in such pieces is always slight.

The smallest pieces in which normal regeneration has been observed were about one twentieth of the body-length and from the region just posterior to the œsophagus. When the regeneration was complete these pieces appeared to consist of little but tentacles and disc, which were only slightly smaller than in pieces many times as large. My observations prove that typical regeneration is possible in very small portions of the body, provided they represent complete transverse or oblique sections of the body; pieces in which the transverse continuity is interrupted roll up in such manner that typical regeneration can never occur.

The Effect of Temperature on Regeneration.

No special experiments in relation to temperature were performed, but my work upon *Cerianthus* extended from September, 1902, to February, 1903. In September the temperature of the water was very high; during October, as the weather became cooler, it fell gradually and continued to do so as long as observed, *i. e.*, until February. A comparison of similar series of pieces begun at different times during this period affords an interesting illustration of the effect of temperature. The various series for which data are given in this paper will serve for this purpose.]

Series 22 was begun September 24; Series 35, October 20; Series 45, November 7; Series 54, 55 and 56, December 15. If the series be considered in this order a considerable decrease in the rapidity of regeneration is noticeable. In Series 22, September 24, the marginal tentacles first appeared in the pieces *A* four days after section. In Series 54, 55, 56, December 15, the marginal tentacles in the pieces *A* from about the same level of the body as Series 22*A* appeared eleven days after section. Between these two extremes lie the other series, and in every case the later in the season the series was begun the less rapid the regeneration. It is not necessary to go over the data in detail to

illustrate this point. In general it may be stated that the rapidity of regeneration in winter during the earlier stages is only about one third of the rapidity in summer.

The amount of regeneration as well as the rapidity also differs with the season. During September and October regenerated tentacles attained a length about twice as great as in similar pieces during January and February.

The length of time during which increase of size in regenerated structures, *e. g.*, tentacles, continues does not differ widely at high and low temperatures, and since regeneration is less rapid it is evident that the total amount must decrease with the temperature.

The most interesting point in connection with the effect of temperature on regeneration is the increase in size of the region at the aboral end in which regeneration does not occur. During the earlier months of my work the only pieces which failed to regenerate were the aboral tips comprising about one eighth of the body-length. Later, as illustrated in Series 45, an aboral piece about one fifth of the body-length failed to regenerate. In December, January and February pieces comprising more than the aboral third failed to regenerate or showed only slight traces of regeneration, as in Series 56*B*. Thus the portion of the body incapable of regeneration increases as temperature decreases.

In general, the effect of temperature upon regeneration in *Cerianthus* is what might be expected, since the activities of living substance in general increase and decrease with the temperature. At high temperatures it is only the extreme aboral end which cannot regenerate typically. From this "inactive" region regenerative power increases toward the oral end. As the temperature falls the limit of "inactivity" must advance toward the oral end, and more and more of the body be included in it.

The Number of Regenerated Tentacles.

It was determined that the number of tentacles regenerated is always less than the original number, and furthermore, that it decreases as the distance between the oral end of the piece and the oral end of the body increases.

It is difficult to determine with certainty the number of tentacles in living specimens of *C. solitarius*, but in fixed material

they can readily be counted. A considerable amount of material fixed for this purpose was lost by accident when too late to replace it, so that exact data cannot be given at present. The decrease in the number of tentacles with increasing distance from the oral end is, however, always noticeable even on cursory examination of the pieces.

Another spieces was also employed for this purpose, viz: the small, whitish, undetermined species mentioned in the preceding paper. In this form the tentacles are much less numerous than in *C. solitarius*, and can usually be counted without difficulty in the living specimen. This species differs somewhat from *C. solitarius* in the extent of regenerative capacity, but the course of regeneration is the same, and since the structure is in general similar to that of the other members of the family, the results obtained from this species may be accepted as typical. In all cases the regenerated tentacles were counted only after they had attained a length of several millimeters; time being thus afforded for the establishment of the small tentacles in the growing region, which sometimes regenerate somewhat later than the others. Only marginal tentacles were counted since the labial tentacles are fewer in number than the intermesenterial chambers and regenerate less regularly than the marginal tentacles. The following series are given as examples:

Series 2, October 7. — Original number of marginal tentacles 21. After removal of disc and tentacles the body was cut into five pieecs, *A*, *B*, *C*, *D*, *E* (Fig. 8). Number of marginal tentacles regenerated *A*, 0, died; *B*, 15; *C*, 14; *D*, 13; *E*, 0, did not regenerate.

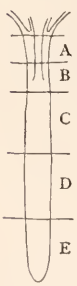

Fig. 8.

Series 5, October 19. — Original number of marginal tentacles 19. After removal of disc and tentacles body cut into five pieces, *A*, *B*, *C*, *D*, *E* (Fig. 9). Number of marginal tentacles regenerated: *A*, 0, died; *B*, 17; *C*, 15; *D*, 13; *E*, 0, did not regenerate.

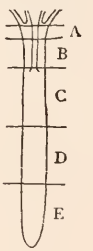

Fig. 9.

These two series are sufficient to show that the number of tentacles regenerated depends on position and has no relation to size of the piece. In both series the piece *B* is only half the size of *C*, yet it regenerates a larger number of tentacles.

These relations hold for all cases recorded, and, since the marginal tentacles correspond to the intermesenterial chambers, they are exactly what might be expected. The number of marginal tentacles which regenerate on a given piece is determined by the number of intermesenterial chambers which it possesses, or the number which it acquires by regeneration of mesenteries. The arrangement of mesenteries in *Cerianthus* was described in the preceding paper. All mesenteries appear first at the oral end and extend gradually in the aboral direction. In general, the younger a mesentery the less its length. Consequently as we pass aborally the number of mesenteries actually present decreases. After section at any level where regeneration is possible a powerful stimulus to growth exists; the mesenteries which extend into the piece remain and become united with the new œsophagus and others regenerate. It appears that the power to regenerate a given mesentery extends for a certain distance aboral to the end of that mesentery and since the mesenteries terminate at all levels we may now conclude that the "determination" of the body-wall for the regeneration of mesenteries shows a similar arrangement. Thus the youngest, shortest mesenteries can be regenerated only within a very short distance from the oral end, the next older and longer within a somewhat greater distance, and so on. The experimental results confirm and establish this conclusion in every case. The addition of new material to the body of *Cerianthus* in producing increase in circumference and number of tentacles and mesenteries may be conceived as a wedge of material which enters the body-wall at the oral end and continually forces its way aborally, thus separating the older parts of the body more and more widely. This conception of the method of growth in the transverse direction agrees with the view expressed above that the general reactive capacity of the tissues is greatest at the oral end. The stimulus to the formation of new mesenteries always becomes effective first at the oral end and from here gradually extends aborally until it is insufficient under ordinary conditions to give rise to a mesentery. Even at this level, however, a mesentery may be formed if the stimulus increases in intensity, or if the tissue becomes more susceptible to it. The resulting conditions give rise to rapid

growth and we may suppose that formative stimuli which were insufficient to produce visible results so long as the piece formed an integral part of the parent-body, either become more powerful after artificial section or else that the new embryonic tissue becomes more susceptible.

A comparative account of the factors of position, size and temperature in *C. solitarius* and the other species studied is rendered unnecessary by the close agreement between all four species. Similar results were obtained in all cases. The only points worth noting here are slight differences in the degree of regenerative power. *Cerianthus membranaceus* regenerates less rapidly than the other species. In the small whitish undetermined species the aboral region in which typical regeneration does not occur is relatively much longer than in *C. solitarius* at the same temperatures. During October only about the aboral fifth or sixth of the body is incapable of regeneration or regenerates incompletely in *C. solitarius*, while in the other species a cut surface only a short distance aboral to the middle of the body is either incapable of regeneration or regenerates incompletely. This species is much less common than *C. solitarius* and was only rarely obtained; no opportunity offered of determining its regenerative power during the colder months. Presumably, however, regeneration would have failed to occur except in the region near the oral end.

One important result of this study may be summed up in the statement that the rapidity and the amount of regeneration in pieces from the body of *Cerianthus* depend primarily on the previous relations of pieces to the parent-body, *i. e.*, on the position and consequent functional condition. This fact is of importance as indicating that the phenomena of regeneration are due not to any special regulatory mechanism which bring about return to a "normal" or "typical" form, but to the same properties which cause growth and differentiation under normal conditions.

Summary.

1. The power of regeneration from cut surfaces in *Cerianthus* is greatest at the oral end and decreases aborally, becoming null a certain distance from the aboral end. This decrease of regen-

erative power is shown by differences both in rapidity and in amount of regeneration.

2. The size of the piece does not influence the rapidity or the amount of regeneration, except in the latest stages. Since the regenerative power is different at different levels, the minimal size of a piece capable of regeneration also differs at different levels, but inversely as the regenerative power.

3. Rapidity and amount of regeneration increase and decrease with the temperature.

4. The assumption of a special regulatory mechanism is not necessary for the explanation of form regulation in *Cerianthus*.

Hull Zoölogical Laboratory,
University of Chicago, July, 1903.

A STUDY OF THE RATE OF REGENERATION OF THE ARMS IN THE BRITTLE-STAR, OPHIOGLYPHA LACERTOSA.

CHARLES ZELENY.

Two interesting internal factors regulating the rate of regeneration of the arms in the brittle-star, *Ophioglypha lacertosa*, were discovered in the course of a study of the problem at the Naples Zoölogical Station during the past winter (1902–03). As some months must elapse before a full discussion of the results can be published it has been thought advisable to give the general data in a preliminary paper. This seems especially desirable at the present time because of the high interest taken in the experimental evidences of a far-reaching correlation between the parts of the individual in both animals and plants.

The experiments to be described give data which show that the rate of regeneration of the arms varies on the one hand with the size of the animal and on the other with the number of removed arms. The first-mentioned correlation gives a maximum rate of regeneration for the medium sized individuals with a pronounced decrease for the smaller as well as for the larger ones. The second correlation, with one exception to be mentioned, gives us an increase in the rate of regeneration of an arm as we pass from the cases with a smaller to those with a greater number of removed arms. The series with all five arms missing is excepted in the statement because the animals in this lot in every instance died or showed evidences of decay before the completion of the experiment.

Method. — Forty-five perfect specimens were divided into five equal groups of nine each, care being taken to distribute them in such a way as to make the sets approximately equivalent as regards size of individuals. The operation consisted in the removal of one or more arms by a transverse cut at the disk level. In the first series one arm was removed, in the second two contiguous arms, in the third three contiguous arms, in the fourth four, and in the fifth five arms. The animals were kept in ten "battery" jars, two for each series, and *were not fed during the whole period*

of the experiment. Measurements of the lengths of the regenerating arms were taken 22, 33 and 46 days after the operation. As stated above the specimens in the series where all five arms were removed did not retain their vitality for a sufficient length of time to allow of comparison with the others and they will therefore be excluded from the following tabulation.

As the rate of regeneration varies with the size of the animal as well as with the number of removed arms the proper relations can best be represented by means of curves. The figure accompanying this paper gives the average of Series I. and II. (those with respectively one and two arms removed) in one curve and of Series III. and IV. (those with respectively three and four arms removed) in the other. The same arrangement is followed for each of the three measurements taken respectively 22 days, 33 days and 46 days after the operation. This brings out the desired relations more clearly than would have been possible if all the individual data had been included. In the figure the abscissæ give the size of the animal as represented by the disk diameter in millimeters. The ordinates give the length of the regenerating arm or arms, also in millimeters. In the series where more than one arm was operated on the regeneration length as given is an average of all the regenerating arms of the individual. As the individual disk diameters are not exactly equivalent in the different series it was found convenient in taking the averages for the combination curves to use arbitrarily disk diameters equal to whole millimeters as the points for comparison. The curves in the figure are therefore constructed on this basis. The unbroken line in each case gives the average of Series I. and II. (one and two arms removed) and the broken line of Series III. and IV. (three and four arms removed).

Statement of Data and Discussion. — The curves show very distinctly the correlation between the rate of regeneration and the size of the animal on the one hand and the number of removed arms on the other.

1. Taking up first the size correlation we find that, starting with the smaller individuals, as we advance toward the larger ones there is a general increase up to a maximum at a diameter of 12 to 15 mm. This is most striking in the two later meas-

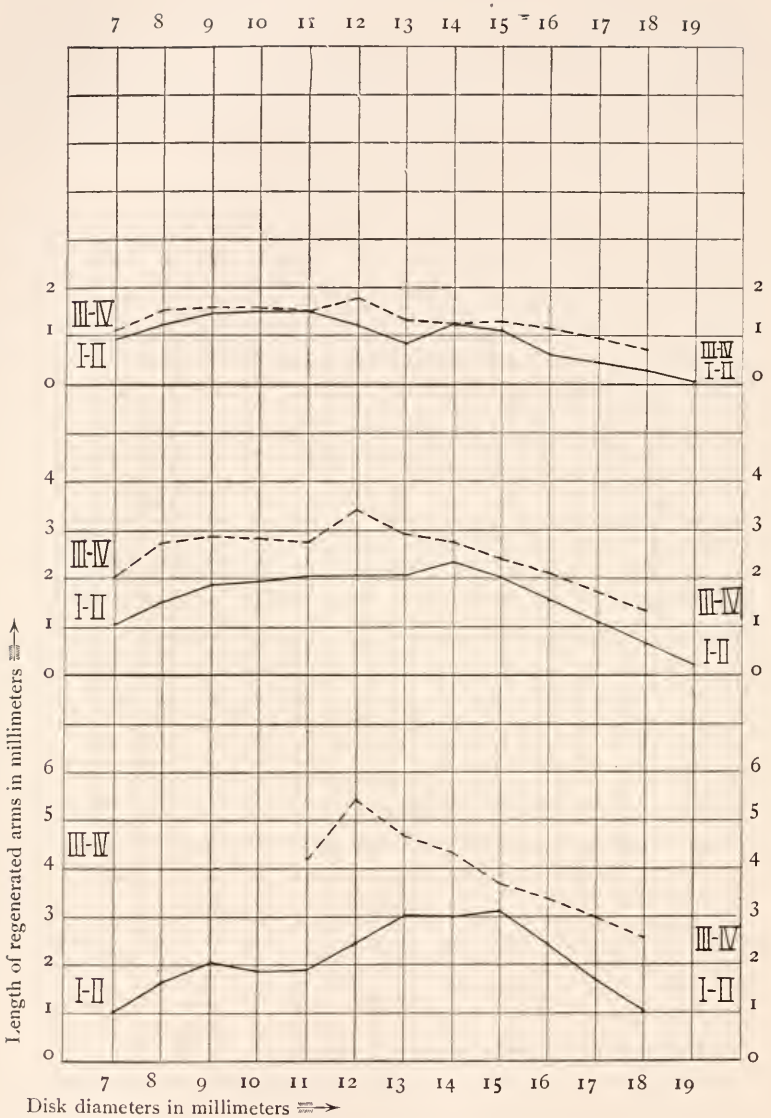

FIG. 1. The abscissæ. represent the disk diameters in millimeters and the ordinates the regenerated arm lengths, also in millimeters. The unbroken line gives the average of Series I.–II. (the specimens with one and two arms removed). The broken line gives the average of Series III.–IV. (the specimens with three and four arms removed). The uppermost curves show the conditions 22 days, the middle curves 33 days, and the lowermost curves 46 days after the operation.

urements taken 33 days and 46 days after the operation. Thus in the 33-day measurement for Series I. and II. the regenerated length increases from 1.07 mm. for a disk diameter of 7 mm. to a maximum of 2.37 mm. for a disk diameter of 14 mm. and then goes down to .21 mm. for a 19-mm. diameter. Also for the Series III. and IV. at the same time the length increases from 2.04 mm. at a diameter of 7 mm. to a maximum of 3.45 mm. at a 12-mm. diameter and down again to 1.36 mm. at a diameter of 18 mm. *The medium sized individuals thus have the maximum rate of regeneration.*

2. More striking still is the very constant difference between the average of Series I. and II. as compared with Series III. and IV. This shows a very decided advantage in favor of the animals with the greater number of removed arms. The difference is evident in the upper curves of the figure from measurements taken 22 days after the operation but becomes more striking in the 33-day and 46-day curves. For example, in the 33-day curve for a 12-mm. diameter (the diameter at which we have the maximum rate of regeneration of Series III. and IV.) we get a regenerated length of 2.08 mm. for Series I.–II. and of 3.45 mm. for Series III.–IV., an advantage of 1.37 mm. or 66 per cent. in favor of the latter. Likewise at a diameter of 14 mm. (where the Series I.–II. has its maximum regeneration) we get 2.37 mm. for Series I.–II. and 2.77 mm. for Series III.–IV., an advantage of .4 mm. or 17 per cent. in favor of Series III.–IV. In a similar manner in the curves obtained from the 46-day measurements we get at a 12-mm. disk diameter a regenerated length of 2.46 mm. for Series I.–II. and 5.42 mm. for Series III.–IV., and at a 15-mm. diameter 3.14 mm. for Series I.–II. and 3.72 mm. for Series III.–IV. which represents an advantage for the group with the greater number of removed arms of respectively 2.96 mm. (= 120 per cent.) and .58 mm. (= 18 per cent.) for the two points named.

We must therefore conclude that when more than one arm is removed the regenerative energy as expressed in the replacement of the lost arms is greatly increased. Not only is the total regenerative energy greater in this case but the energy expressed in each arm is greater than the total energy when only one is removed.

Expressing this in mathematical form, if E_1 represents the regenerative energy exhibited in the replacement of the lost arm when only one is removed, assuming that increase in length is a measure of such energy, and E_n represents the energy exhibited in regeneration when more than one arm is removed, n being the number of absent arms, then not only is $E_n > E_1$ but also $E_n/n > E_1$ or $E_n > nE_1$. Therefore when we remove n arms we increase the total regenerative energy by more than n times the amount exhibited when only one is removed. The force of this statement is made especially strong when we consider that throughout the experiments the animals received no food supply whatever.

Expressing the relation in still another way, let us take a brittle-star with arms A, B, C, D and E, in which a_1, b_1, c_1, d_1 and e_1 represent the respective lengths these arms will attain after a definite period of regeneration, supposing that one alone is cut off in each case. Now let us suppose instead that the first four are cut off, then after this same period of time we get for the regenerated lengths $a_4 > a_1$, $b_4 > b_1$, $c_4 > c_1$, $d_4 > d_1$. Now in the first case mentioned we cannot assume that the stimulus of removal and the resultant reaction of regeneration are purely local and concern only the tissues in the immediate vicinity of the cut surface for we then get into difficulty as soon as we try to explain the cases where four arms are simultaneously removed. Here we find we must add a considerable quantity (r_4) to each of the original single regeneration lengths, *e. g.*, $a_4 = a_1 + r_4$. Then $a_4 + b_4 + c_4 + d_4 = a_1 + b_1 + c_1 + d_1 + R_4$ where $R_4 (= \Sigma r_4)$ represents the total response of the organism as a whole which must be added to the local effects of the operation stimulus. If, on the other hand, we consider the influence of the organism as a whole on the regeneration of its arms as one of retardation, we must take the values a_4, b_4, c_4 and d_4 as representing most nearly the original local stimulus effect. Then without changing the values of r_4 or R_4 we may rearrange the formulæ, making $a_1 = a_4 - r_4$, etc., and $a_1 + b_1 + c_1 + d_1 = a_4 + b_4 + c_4 + d_4 - R_4$.

But whether we consider the influence of the organism as a whole to be one of acceleration or one of retardation we must recognize in either case that the regeneration rate is not a matter which involves only the local conditions at the wounded surface

as determined by the direct action of the operation. It seems, on the other hand, to be bound up with intricate reactions affecting the whole character of the activities and organization of the animal. A more direct application of the above statements to the special theories of regeneration would be out of place at the present time.

We may sum up my results on the rate of regeneration of the arms of the brittle-star, *Ophioglypha lacertosa*, as follows :

1. There is a definite relation between the size (*i. e.*, age (?)) of the animal and the rate of regeneration of its arms. The maximum rate is exhibited by individuals of medium size (with a disk diameter of 12 to 15 mm.). Both the smaller and the larger ones give a diminishing rate as we go away from this point.

2. The greater the number of removed arms (excepting the case where all are removed) the greater is the rate of regeneration of each arm.

Hull Zoölogical Laboratory,
The University of Chicago,
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COMPARATIVE VARIABILITY OF DRONES AND WORKERS OF THE HONEY BEE.[1]

D. B. CASTEEL AND E. F. PHILLIPS.

Introduction.

According to the theory of germinal variation it would be concluded that the workers of the honey bee, *Apis mellifica*, being produced from fertilized eggs, would show more variation than would the drones which come from parthenogenetic eggs. This variation would be manifested by coloration and by relative size of parts, and it might be expected that a series of measurements made on like parts of drones and workers would show a smaller degree of variability for drones than for workers. To test this fact a series of measurements have been made and the results tabulated.

Owing to the difficulty of measuring the extent of coloration on the segments of the abdomen this could not well be used for this work, and so a series of measurements on the wings were chosen although coloration is practically the only difference usually observed between the varieties of *Apis mellifica*.

The wings are also desirable for other reasons. They are of classificatory importance in systematic work, do not shrink when preserved in alcohol and are easily examined with a microscope by simply clipping off the wing and mounting in alcohol on a slide. They also give more accurate results since the extent of coloration would vary according to the retraction of the segments of the abdomen in preserving and it would be practically impossible to get the individuals normally extended in all cases.

The reason for taking up this work was rather indirect and should perhaps be stated since the results throw some light on a widely separated line of work. Perez[2] (1878) took an Italian queen fertilized by a French black drone, and after some time examined 300 drones from this queen. As the queen was pure

[1] Contribution from the Zoölogical Laboratory of the University of Pennsylvania.

[2] Perez, J., "Mémoire sur la ponte de l'abeille reine et la théorie de Dzierzon." *Ann. Sci. Nat.*, 6 Sér., Zoöl., T. 7, 1878.

Italian her drones would also be pure Italian, since they are produced from parthenogenetic eggs, and the fact that she was mated with a black drone should make no difference. He found, however, that 149 of the drones did show some markings which he thought indicated hybridism, and from these observations rejected the theory of Dzierzon. His results were criticised severely and all manner of arguments were used against them, atavism, impurity of the queen and other reasons being given in explanation. Weighing the arguments of Perez and those presented in opposition to them, however, would lead one to believe that Perez had the best of the argument. If then we accept the theory of Dzierzon, and it is well established, we must account for the results of Perez.

An examination of a large number of hives has shown us that the coloration of the drones cannot be used as a test of their purity, and that, therefore, Perez' work is inaccurate, since he used this test as the basis of his argument. Drones from an Italian queen fertilized by an Italian drone show gradations in amount of coloration of the segments of the abdomen which would easily lead one to conclude that some of them were not pure, provided the evidence for their purity was not so strong; while at the same time the workers from the same queen show a uniformity of marking which is very striking. In the face of these facts it is evident that extent of coloration could not be used as a basis for investigation in relation to parthenogenetic development in the case of the bee. It might also be added that the fact of the irregularity of coloration of the drones is well known to most bee-keepers, and a number of these men have stated to us that they do not consider the coloration of the drones as in any sense a test of purity.

This little investigation led to the conclusion that possibly the drones showed more variation in other ways than did the workers, and to test this the measurements here recorded were made.

We wish to express our appreciation to Mr. E. L. Pratt, of Swarthmore, Pa., for material furnished, and especially to Mr. E. R. Root, of Medina, Ohio, for material and for many courtesies shown during investigations carried on by one of us in his apiary.

Measurements.

For the measurements taken in this work we have chosen veins and cells which in the bee differ from the typical hymenopterous wing and which are to a certain extent typical of the bee in their direction and extent. In this choice we have followed the discussion of the venation of the Hymenoptera of Comstock and Needham.[1] The measurements were: (1) Length of vein radius (R); (2) diagonal length of cell radius-four (R_4); (3) length of vein media-two (M_2); (4) length of medial cross-vein (m); (5) ratio between m and M_2, and (6) number of hooks or hamuli on the hind wing. An attempt was made to measure the angles formed by the union of veins radius-four (R_4) and radius-sector (Rs), and veins radius-five (R_5) and radius-sector (Rs), but on account of the difficulty of getting the exact angle at which the veins branch these measurements were discontinued through fear of inaccuracy. In all cases right wings

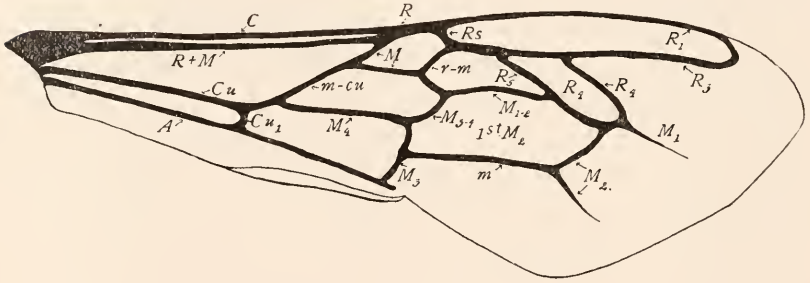

Fig. 1.

were measured. The measurements were made from camera-lucida sketches, Leitz ocular 2, objective 3 with lower lens removed and sketch made at table level, this giving a magnification of forty-two diameters. In all cases this magnification is retained in the tables.

The choice of the veins and cells measured perhaps needs some explanation since each one has certain peculiarities in direction or extent. It should be stated, however, that we do not think it makes much difference what veins are chosen for a comparison of variations in this case since all our observations show

[1] Comstock, J. H., and Needham, J. G., "The Wings of Insects," Chap. III. (continued), IX., "The Venation of the Wings of Hymenoptera." *Amer. Nat.*, Vol. 32, pp. 413–424, 12 figs., 1898.

about the same degrees of variability of the two sexes. Any other veins or cells would no doubt show like variations.

Length of Vein R. — In the typical hymenopterous wing the media (M) branches from the vein $R + M$ at a point nearer the base of the wing than in *Apis*. The length of the vein $R + M$ would therefore be desirable for measurement, but from the difficulty of getting exact measurements this was discarded, and in its place we took the measurement from the point where M branches off from $R + M$ to the point where R divides into R_1 and Rs or the length of R, which is therefore shorter than in the typical hymenopterous wing.

Diagonal Length Cell R_4. — In the typical hymenopterous wing veins R_4 and R_5 are nearly at right angles to the vein from which they branch, while in the bee, R_4 is bent out to about 135° and R_5 to 160°. This makes the cell R_4 considerably longer, and the diagonal length varies according as the angle $R_4 - Rs$ varies. The measurements were made from the proximal side of $R_5 - Rs$ to the anterior angle of $R_4 - M_1$.

Length of Veins M_2 and m. — In the bee's wing there is a bending in of the veins M_4 and M_3 toward the base of the wing with a corresponding lengthening and shifting of vein m. This vein gives a convenient measurement for the relative length of wing since it varies almost directly as the length increases. M_2 was chosen because it is correlated in its length with m, and forms a convenient relative measurement for the breadth of the wing.

Ratio between m and M_2. — As stated above, the lengths of m and M_2 are correlated in their variation, so in order to test the relative variabilities of the two veins in drones and workers, we computed the ratios between the two — $M_2 : m :: 1 : x$; x in every case being carried to two decimal places. From these computations it was found that the variation of m is in inverse proportion to that of M_2 as will be shown later.

Number of Hooks on Hind Wing. — This count was taken to see whether the hind wing varied as did the fore wing, and the number of hooks served as a conservative test.

Besides these measurements and calculations we looked for cases of abnormal wings in which the subcostal (Sc), radius-two (R_2) and cubital-two (Cu_2) veins might be present, these

being absent normally in the bee, and also for all other cases of abnormalities in the venation. In none of the wings observed were *Sc*, R_2 or Cu_2 present. The other abnormalities will be discussed later.

The Choice of Material.

The individuals used were not all taken from the same hive, since observations show that all colonies do not vary to the same extent, at least in coloration. For this reason it appeared best to use individuals from different hives and different strains in order to get a more correct idea of the natural variations. In all cases individuals were selected at random. The material used was as follows :

Drones.

I. Fifty individuals from Medina, Ohio, May 16, 1903. Hybrids, Italian and black.

II. One hundred individuals from Medina, Ohio, May 23, 1903. Italians (?) from a peculiar strain bred by F. A. Hooper, Jamaica, very light in color.

III. One hundred individuals from same hive as I. May 25, 1903.

IV. One hundred individuals from Medina, Ohio.

V. Fifty individuals from Medina, Ohio, May 9, 1903. Italians.

VI. One hundred individuals from Swarthmore, Pa., August 20, 1903. Italians from a peculiar strain bred by E. L. Pratt, the queen having an entirely yellow abdomen.

Workers.

I. Fifty individuals from same hive as Drones V., May 9, 1903.

II. Three hundred and fifty individuals from Philadelphia, Pa., August 10, 1903. Italians.

III. One hundred individuals from Philadelphia, Pa., May 15, 1902. Hybrids, Italian and black.

Length of Vein *R*.

The measurement of the length of this vein was found to be somewhat difficult owing to the hairs covering the angles and to the difficulty of getting the exact middle of the curve at the place where R_1 and *Rs* separate. However, with considerable care and

the reëxamination of cases showing the greatest variation we think the figures are nearly correct. Since the drones varied over 5 mm. on an average more than the workers and any error in measurement could scarcely be more than 1 or 2 mm. we feel justified in concluding that in this case the drones vary considerably more than the workers. The greatest variations were in Lot III. of the workers, where the variation was from 32 mm. to 42 mm., and in Lot VI. of the drones, where the variation was from 35 mm. to 48 mm. Lots I. of the workers and V. of the drones taken from the same hive at the same time show a range of variability in the ratio 8:11.

TABLE I.

VEIN *R*.

Lot.	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Av.
Drones.																				
I.					1	1	2	2	3	12	6	12	1	5	3	2				40.16
II.	1	1	1	7	8	22	19	21	11	6	3									35.99
III.					2	3	1	2	5	13	13	12	10	14	12	3	6	3	1	41.42
IV.	2	2	14	16	23	15	13	8	5	1	1									34.38
V.			1	3	3	5	11	8	7	5	3	3	1							36.92
VI.						3		9	11	18	14	10	14	8	8	3		1	1	40.42
	3	3	16	26	37	49	46	50	42	55	40	37	26	27	23	8	6	4	2	
Workers.																				
I.				2	1	3	4	11	17	9	3									37.46
II.					4	13	21	65	78	70	71	17	9		2					38.42
III.			1	1	5	11	10	26	19	12	10	2	3							37.40
			1	3	10	27	35	102	114	91	84	19	12		2					

DIAGONAL MEASUREMENT OF CELL R_4.

This measurement being taken in one of the most variable regions of the wing shows a remarkable difference in the range of variability of the two sexes. The measurement of this distance was quite easy since the limits are well marked and easily distinquishable and in no case do we think there was room for doubt in the length to more than 1 mm. This fact taken with the length makes these measurements a very good test of the relative variability. In this region of the wing a very large part of the abnormalities were found, so that we conclude that this portion shows more variation than any other; but in spite of this tendency

to vary, the workers' wings were quite constant. The greatest range of variation in workers was found to be 13 mm., while the least range in drones was 19 mm. The fact that the average length of the cell in drones was between 96 mm. and 102 mm., while the average length for workers was about 75 mm., will however account for part of this greater range since, with a given range of variability, the greater the length, the less the actual variation. This, however, does not account for the extreme difference which we find and in this case again, as in the first set of measurements, we find a much greater variability in drones than in workers.

Table II.

Diagonal Cell R_4.

Drones.

Lot.	75[1]	80[1]	87[1]	89	90	91	92	93	94	95	96	97	98	99	100
I.				1			1		2	2	2	2	3	4	6
II.				1	2	6	10	9	6	10	6	5	9	8	13
III.							1		1		1	3		6	7
IV.					7	6	5	8	5	13	13	11	8	5	7
V.									1	2	1	3	1	5	4
VI.	1	1	1			1	1	2		12	11	4	6	12	13
	1	1	1	2	9	13	18	19	15	39	34	28	27	40	50

Lot	101	102	103	104	105	106	107	108	109	110	111	112	113[1]	115	Average.
I.	2	2	3	3	4	1	5	3	3	1					101.68
II.	9	4		1			1								96.49
III.	7	8	9	9	11	10	5	8	4	6	1	1	1	1	104.14
IV.	2	4	3	1	1			1							96.15
V.	3	6	6	8	4		2	3				1			101.98
VI.	11	6	8	2	3	2	1	1	1						98.77
	34	30	29	24	23	13	14	16	8	7	1	2	1	1	

[1] No individuals found in columns omitted.

Workers.

Lot.	69	70	71	72	73	74	75	76	77	78	79	80	81	82	Average.
I.	1	1	3	6	6	12	7	3	6	3	1	1			76.36
II.	1	10	18	20	42	37	71	62	38	24	16	4	4	3	75.08
III.		1	1	3	5	8	19	20	12	12	16	2	1		76.24
	2	12	22	29	53	57	97	85	56	39	33	7	5	3	

Length of Vein M_2.

This is not a specialized vein, and while it has for its anterior boundary the edge of the cell R_4, it shows comparatively little abnormality. A few cases of extra veins thrown in in the region of this vein will be discussed later, but it is located out-

side the portion of greatest abnormality of the wing. The principal reason for the measurement of this vein was to get a ratio with the vein *m*, but as the actual measurements add to the evidence, it seems advisable to give the table. The greatest range of variability in workers was 9 mm., the least for drones 11 mm., or, if we drop the two quite abnormal cases in Lot V., 9 mm. Taking into consideration the relative lengths of the average veins, 34 5 mm. and 45 mm., this makes the greatest variation in workers about equal to the least variation in drones, while the greatest range in drones is over twice that of the greatest range in workers.

TABLE III.

VEIN M_2.

Drones.

Lot.	32	33	34	35	36	37	38	39	40	41	42	43	44
I.											3	2	
II.							6	8	12	6	21	16	17
III.													1
IV.			1							3	3	15	9
V.								2					4
VI.								1	1	2	1		2
			1				6	11	13	11	28	33	33

Lot.	45	46	47	48	49	50	51	52	53	54	55	56	Average.
I.	2		8	2	5	15	8	2	2	1			48.82
II.	6	5	2	1									42.26
III.	3	6	7	17	15	25	11	8	1	3	2	1	49.43
IV.	15	13	15	14	6	3	2	1					45.72
V.	8	5	6	10	11	2	1	1					46.96
VI.	2	3	7	11	14	13	10	15	13	2	3		48.64
	36	32	45	55	51	58	32	27	16	6	5	1	

Workers.

	32	33	34	35	36	37	38	39	40	
I.	7	12	19	9	1	2				33.82
II.	8	40	64	134	59	31	10	3	1	34.83
III.	4	10	42	22	10	11	1			34.61
	19	62	125	165	70	44	11	3	1	

LENGTH OF CROSS-VEIN *m*.

This vein shows no abnormalities and may therefore be considered as entirely outside the region so well marked about the cells R_4 and R_5, which shows so much abnormal variation. If it be argued that, since the more anterior veins tend to be abnormal,

they are not therefore good tests of the comparative variability, then, since vein *m* is entirely outside this area, this measurement would serve as an answer to that argument and is in itself a sufficient test. As in the case of vein M_2, the measurements of this vein were made principally for the sake of getting a ratio of variation between two veins which meet at nearly a right angle, but as the drones show greater variation than the workers, although not to so great a degree as in some other cases, and as this is a constant vein, we add the table. The greatest range in workers is 16 mm., the least in drones 15 mm.; the average for workers almost 13 mm., the average for drones 20 mm.; the greatest for drones 23 mm. The average lengths in the two cases (72 mm. and 97 mm.) reduces this difference about one third, so that the least variable drones are about as constant as the least variable workers, but the average range for drones still remains considerably greater than for workers.

Table IV.

Vein *m*.

Drones.

Lot.	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
I.							1				1				1	3	3	1	6
II.										1			4	3	7	12	8	17	8
III.																		2	2
IV.	1		1		4	1	2	3	2	11	11	6	16	5	12	7	8	5	3
V.													2		2	3	4	2	4
VI.				1		1				1	2	2	5	5	6	10	12	14	13
	1		1	1	4	2	3	3	2	13	14	8	27	13	28	35	35	41	36

Lot.	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	Avg.
I.	1	4	2	7	5	2	3	3	3	4										100.04
II.	14	8	5	6	3	2	2													95.65
III.	4	9	5	16	4	12	8	11	10	4	2	4	2		2	1	1		1	102.43
IV.		2																		88.84
V.	9	13	1	3	2	3	1	1												97.08
VI.	6	7	7	5	2	1														94.63
	34	43	20	37	16	20	14	15	13	8	2	4	2		2	1	1		1	

Workers.

	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	Avg.
I.					1	2	2	4	6	13	7	8	4	2	1		72.30
II.	1			2	3	15	12	69	35	73	42	43	40	11	3	1	72.00
III.					1	2	1	10	16	18	27	13	8	2	2		72.43
	1			2	5	19	15	83	57	104	76	64	52	15	6	1	

Ratio Between M_2 and m.

To get this ratio the length of the vein m was divided by the length of the vein M_2 and the division carried to two decimal points giving a ratio in the form $M_2 : m :: 1 : x$. To get the comparative variability of the two sexes the individuals were tabulated according to the values of x and in this tabulation the values of x to *two* decimal points were used, but owing to the length of such a table we have combined these values and give them according to x carried to *one* decimal point. This is advisable also for another reason; owing to the relative smallness of the numbers in the first part of the proportion certain columns remained empty since, for example, no combination of figures between 32 and 57 and between 63 and 115 can give a ratio of 1 : 1.99. In order then to get a series of numbers which represents what is no doubt more nearly the true scale of ratios, the first table has been reduced to a table with x carried to but one decimal point. In tabulating these ratios the individuals were not grouped according to lots as in the other cases since we found practically little difference between the lots in any one sex. The average ratio for drones is 1 : 2.06 and for workers 1 : 2.08 so that no account need here be taken of the difference in averages since it is so slight. The range of the values of x for workers is from 1.70 to 2.34 or .64; that for drones from 1.68 to 2.90, or, omitting one wing with a very abnormal ratio, from 1.68 to 2.57 or .89 showing .25 greater range in drones than in workers. It might also be said, since our long table cannot be used here, that this greater range is not caused by a few abnormal cases but that the variations are shown by a gradually decreasing number of individuals in each value of x in both drones and workers, barring, of course, certain values of x which as above mentioned are impossible with the figures worked upon.

Table V.

Ratio Between M_2 and m.

Value of x.	1.7	1.8	1.9	2	2.1	2.2	2.3	2.4	2.5	2.6
Drones.	13	51	81	126	89	70	36	19	12	3
Workers.	1	11	53	119	217	81	17	1		

The working out of these ratios brought to light another point which explains this greater range in the values of x in the proportion. It was found that a certain inverse ratio exists between the lengths of m and M_2 so that the area of the cell $1st M_2$ bounded distally and posteriorly by these veins remains more nearly constant for wings of the same area than do the bounding veins. This does not mean that the area of the cell $1st M_2$ does not vary, for a little calculation shows that it does vary considerably. The ratios of the wings for each length of M_2 were gathered together and an average taken of the ratios in each case, with the result that we found a relatively constantly decreasing ratio with the increase in the length of M_2. The range of these ratios for drones was from 1.80 to 2.57, omitting again the one abnormal wing mentioned above, and for workers from 1.77 to 2.21, a difference in this case of .33 in the ranges of the two sexes. This would indicate seventy-five per cent. more range of variation for the drones than for the workers and this difference is directly correlated with the great difference in the size of M_2 found to exist.

TABLE VI.

RATIOS FOR LENGTHS OF VEIN M_2.

mm.	32	33	34	35	36	37	38	39	40	41	42	43	44	
Drones.			2.05				2.57	2.43	2.35	2.25	2.28	2.16	2.14	
Workers.	2.21	2.19	2.13	2.06	2.01	1.93	1.90	1.86	1.77					

mm.	45	46	47	48	49	50	51	52	53	54	55	56	Av.
Drones.	2.09	2.06	2.02	2.03	1.98	2.00	1.91	1.88	1.83	1.80	1.79	1.80	2.06
Workers.													2.08

HOOKS ON THE HIND WING.

The number of hamuli or hooks on the hind wing which are used to fasten the two wings together during flight were used as a means of testing the variability of this wing. The examination of 1,000 wings has shown that this is not the most variable feature of this wing, but that far more variation occurs in the breadth of the wing and in the angles of the veins which form the cross supports. As the area of the hind wing increases the increase takes place principally by a widening of the wing although by no means entirely by that method. The drones especially show

this widened wing, in some cases the width equalling the length. It may safely be stated, although no measurements were made on this point, that the area of the back wing is more variable than that of the fore wing. Since the number of hooks is correlated with the length of the wing they do not, therefore, show as much variation as would be found on the other parts of the hind wing. The least range of variation in number of hooks in workers was over seven points, the greatest, ten; the least for drones, nine, the greatest eighteen, or omitting one very abnormal wing with but twelve hooks, twelve. The relative amounts of variation are

FIG. 2.

shown far more clearly here by examining the numbers of individuals in each case which have the same number of hooks. For the workers the greatest number is 139 individuals which have 21 hooks while for drones the greatest number is 98 with 22 hooks, the average number for drones being a little higher than for workers. In workers the descent in numbers of individuals from 21 is far more sudden than that for drones, which is really

TABLE VII.

HOOKS ON HIND WING.

Drones.

	12	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Avg.
I.				4	4	8	11	9	4	6	1	1	2			21.56
II.		2	1	12	21	23	20	17	2	2						20.12
III.				1	7	12	15	28	14	14	6	2	1			22.09
IV.			4	10	17	22	27	12	2	5	1					20.33
V.				4	2	11	10	9	7	1	4	1		1		21.54
VI.	1			3	3	7	6	23	23	19	6	6	1		1	22.42
	1	2	5	34	54	83	89	98	52	47	18	10	4	1	1	

Workers.

I.					6	9	12	11	6	5	1					21.42
II.			4	13	42	74	94	61	45	11	4	2				21.08
III.			2	11	18	18	33	8	6	4						20.37
			6	24	66	101	139	80	57	20	5	2				

the true test of the relative variability far more than is the range of variation.

So far we have discussed the range of variability in each case, but a far more important test of the comparative variability is the relative centralization of individuals about the average point in the table. If, for example, the drones showed exactly the same range as did the workers, the fact that in every one of the tables the workers show a greater number of individuals with the average dimensions, together with a rapidly decreasing number in each direction, while the drones show a more nearly equal number of individuals over several dimensions on each side of the average point, would lead one to conclude that the drones show far more variability than do the workers. It is not necessary to go over all the tables to point out this fact, but even a hurried examina-

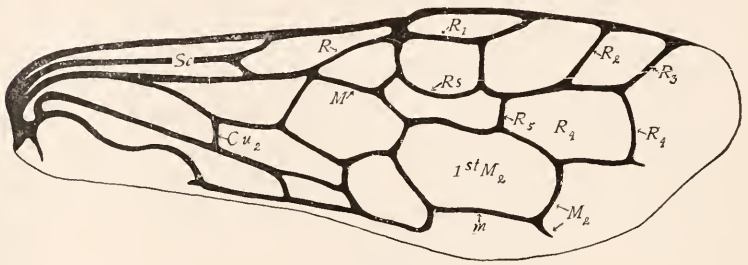

FIG. 3.

tion will show the facts as above stated. If, for example, the tables were put in the form of curves of variation the workers would show a longer and narrower curve in every case than the drones, and were these curves expressed in the form of mathematical formulæ the fact would be brought out still more strongly that the males in this case show more variation than do the abortive females.

It may be well here to offer some explanation as to why curves were not used in this work, since that is the usual method for work on variation. It was not the purpose of this investigation to work out the law of variation for the two sexes, but merely to show which sex did vary to the greater extent. This purpose has been fulfilled and is shown in the tables. In order to get a true curve of comparative variability it would be necessary to

measure many times the number of individuals which were used and to extend the observations over a far greater range of varieties. No curve made with 500 individuals would express the true law of variation, nor would ten times that number be sufficient, and since the formulation of the law of variation for parthenogenetic and fertilized forms for this particular kind of parthenogenesis, arrenotoky, is too important a matter to be based on an inaccurate mathematical formula, it seems better to us to state simply the fact that greater variation does occur in the males and leave the formulation of the law to be worked out with a far greater range of observations and measurements. And then, too, it is by no means certain that this variation follows any fixed law. If the variation were caused entirely by germinal variation, or by any other one factor, then it might be assumed that the law of this variation could be stated in the form of a mathematical formula, but as will be shown later, it appears probable that a large part of the greater variability of the drones is due to chance and is therefore not in accordance with any law. It may be argued that variation according to chance is but a way of stating our ignorance of the true law, but if there is a law for this variation it is certainly very obscure, and the working out of this law would require an extremely large number of measurements taken from individuals, each one with its life history known together with a high degree of mathematical ability in its formulation.

Abnormal Wings.

As noted previously, in all wings examined, record was made of wings having veins which are not typically found in the bee. Fig. 4 shows, in dotted lines, where these abnormalities occur most frequently. It is very difficult to record these irregularities in any kind of a table, since the irregular veins vary widely in extent and do not arise at exactly the same place in many cases. An attempt was made to classify these according to the veins from which they branch, their extent and direction. Manifestly any tabulation must be considered as merely a matter of convenience in examination. In this we have recorded cases where a vein bends (b in table) from its true course, showing but a tendency toward abnormality, as well as the well-marked cases. The ex-

tent of the abnormality is expressed roughly in the terms, very small (vs), small (s), almost complete (ac) and complete (c). The letters used to designate these abnormalities are in no way connected with the naming of the normal veins, but are chosen merely as a convenient means of marking the irregularities. It will be understood that it is impossible to draw at all times the same line between, for example, the terms small and very small,

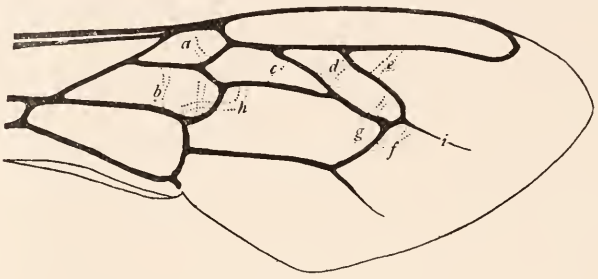

FIG. 4.

and the table is of no value except to give an idea of the number of cases of abnormalities.

	MALES.	FEMALES.
a.	82 b, 32 vs, 109 s, 1 ac, 2 irregular.	2 b.
b.	7 s, 1 double s, 4 ac, 6 c.	2 b.
c.	3 vs, 18 s, 2 ac, 13 c, 2 irregular.	1 b, 3 vs, 4 s.
d.	11 b, 27 s, 1 double s, 7 ac, 2 c.	3 b, 6 vs, 9 s.
e.	9 s, 1 ac, 5 c.	1 b, 1 vs, 3 s.
f, *g*.	8 irregular at anterior end.	2 irregular at anterior end.
h.	5 s, 2 c, 1 very irregular.	none.
i.	none.	2 lost veins ; 1 almost lost.
Other irregularities, 14.		none.

Deducting the cases where more than one irregularity occurs on one wing we have 271 irregular drone wings and 37 worker wings. Leaving out of consideration those cases in which merely a bend is recorded there are 206 irregular drone wings and 30 irregular worker wings or almost seven times as many for drones as for workers.

The figure and these tabulations make clear what has been said previously about an area of irregularity in the wing. This region is rather well defined and no irregularities in venation were seen outside it.

A point which may well be recorded is that none of these veins

could be considered as in any sense mutations since there are in all cases varying degrees of abnormality indicating a gradual variation in the directions indicated.

No attempt has been made to correlate definitely these abnormalities with any lengths of the neighboring veins, but in a general way it can be said that the largest wings of each sex are the most abnormal, and this fact explains to some extent why the drone wings are so much more abnormal than those of the workers. From this it would appear that the cause of these abnormalities was the need of extra strengthening of the cells as they became larger, and that the irregularities are the result of extra growth energy, which has a chance to show itself when room is allowed for its manifestation.

Some Explanations of These Results.

With these facts before us it seems desirable to find, if possible, some explanations for these peculiar results. As stated in the introduction the theory of germinal variation would lead one to believe that the parthenogenetic individuals of the bee would show less variation than those from fertilized eggs and if there were no complicating factors it may be supposed that this would be true, but there are certain factors which modify this result so that the ratio of variation is exactly reversed.

In the first place that kind of parthenogenesis in which males only are produced (*arrenotoky*), is not such a specialized form of agamic reproduction as are those kinds of parthenogenesis in which females or both males and females are produced. When females only result from parthenogenetic eggs (*thelytoky*), a crossing of two lines of heredity seldom occurs and amphimixis can bring about little variation. In the case of the Aphids and Daphnids we have a similar condition except that once in a year or once in some life cycle males also are produced parthenogenetically and then there is an opportunity for the blending of hereditary traits through fertilization. To this last form of parthenogenesis the name *amphoterotoky* is applied. It is thus seen that in arrenotoky this mixing of hereditary traits is not dispensed with to such an extent as in the cases of thelytoky and amphoterotoky. On the other hand, the production of females par-

thenogenetically is of far more use to a species where gamic reproduction is unnecessary, since fertilization is not necessary to give a stimulus to the egg so that it may develop, and what the species loses in lack of cross fertilization is more than made up in the advantage it has through its parthenogenetic power. Since then arrenotoky is the least specialized form of parthenogenesis, and since a crossing does occur at every *second* generation in species with this power, it follows that according to the theory of germinal variation we would find more variation in arrenotoky than in either thelytoky or amphoterotoky. In fact the decrease in variability would not be very great, since in every case but one half the crossing is dispensed with.

Variation in parthenogenetic forms has been observed previously. Weismann[1] found that the parthenogenetic ostracod, *Cyprus reptans*, showed variation, and Warren[2] found considerable variation in Daphnids. Both of these cases fall under amphoterotoky, so that on *a priori* grounds we would expect still more variation in arrentoky. It is also held by some that males tend to vary more than females, and perhaps this tendency has something to do with what we find in the case examined. Davenport and Bullard,[3] found 2½ per cent. more variation in males than in females in swine. Darwin[4] gives a considerable number of cases showing the same tendency, and others have observed similar facts. On the other hand there are cases in the Odonata (*Gomphus* and *Macrothemis*, Calvert[5]) and in the Lepidoptera (*Thyreus abbotii*, Field[6]), in which the females are more variable than the males so that we must not assume too much on this ground.

This still leaves considerable variation in drones to be accounted

[1] Weismann, A., "The Germ-plasm," 1893.

[2] Warren, E., "An Observation on Inheritance in Parthenogenesis," *P. R. Soc. Lond.*, Vol. LXV., pp. 154–8, 1899.

[3] Davenport, C. B., and Bullard, C., "Studies in Morphogenesis," VI. "A contribution to the quantitative study of correlated variation and the comparative variability of the sexes." *Proc. Am. Soc.*, Vol. 32, pp. 85–97, 1897.

[4] Darwin, Charles, "The Descent of Man." London, 1871.

[5] Calvert, P. P., "The Odonate Genus *Macrothemis* and Its Allies," *Proc. Boston Soc. Nat. Hist.*, Vol. 28, pp. 301–332, 1898. "On *Gomphus fraternus*, *externus* and *crassus* (Order Odonata)," *Entomol. News*, March, 1901.

[6] Field, W. L. W., "A Contribution to the Study of Individual Variation in the Wings of the Lepidoptera," *Proc. Am. Ac. Sc*, Vol. 38, pp. 389–396, 1898.

for and the following facts seem to us to help in this. The workers in a hive are hatched from cells one fifth of an inch in width and the size of these cells is remarkably uniform. On the other hand the drones hatch from cells which are generally one fourth of an inch in width, but often hatch in worker cells and from cells of all intermediate sizes. In the making of the comb under natural conditions there are a great many irregular cells formed which are transition cells between the worker and drone cells, and from these, if used for brood at all, drones are produced. It is true that sometimes a worker is produced in a drone cell, but this is very rare, provided there are any worker cells in the hive. Drone pupæ, on the contrary, are frequently seen in worker cells and are very noticeable on account of the exceptionally high arched cap which the workers put on when the larva is sealed up. This then gives to drones a greater amount of variation in the room provided for their growth while in the plastic state.

A bee larva will grow until it fills the cell in which it is placed and the young bee which emerges will be the size of the cell from which it came, within certain limits. This is shown in the production of queens by the modern methods of queen rearing used in apiculture. A young worker larva, less than one day old, is lifted from its cell and put into a cell cup of queen size. The workers complete this cup and form a queen cell and the larva in this cell grows to a much larger size than would be possible if it had remained in its original cell. Once in a while the bees will attempt to make a queen from a drone larva and while, of course, this is a failure yet the result is a very large drone. Generally, however, the drone under these conditions dies before reaching the imago stage. These facts show that the growth of an individual is limited by the size of the cell and also undoubtedly by the amount of food received during the unsealed larval stage. Then it follows that since the cells from which drones hatch vary from one fifth of an inch to over one fourth of an inch in width, while those from which workers hatch are quite uniform, that the variation in size will be considerably greater for drones than for workers.

This supposition is further strengthened by some of the facts

brought out by measuring the wings. Referring again to the table of the ratios varying according to the length of vein M_2 we find that the vein *m* varies *inversely* as the length of M_2. The length of vein *m* represents the ratio of the length of the wing while vein M_2 represents the ratio of the width. Probably most of the drones which have the shortest vein M_2 are those hatched from the smallest cells and the wings could not increase in width and therefore to meet the needs of the animal in making the necessary area of wing for flight the vein *m* must be lengthened. On the other hand, those drones hatched from the largest cells would be allowed greater room for the development of vein M_2 and vein *m* need not be so long.

Another fact which seems to indicate this is that those drone pupæ which are developing in worker cells are covered over by a very high cap, making the length of their cell much greater than that of the ordinary drone cell. The drones which hatch from these cells are long and narrow when compared with those from drone cells proper.

The drones in Lot II. were taken from a hive in which there were no drone cells except possibly a very few in the corners of the frame or near the top bar of the frame since all the combs were made on what beekeepers call foundation and the cells were uniformly of worker size. These drones show the least variation since they were all hatched under the same conditions. The drones of Lots I. and III. were hatched in old irregular combs and the tables show considerably greater variability.

The greatest number of abnormalities were found on the largest drone wings and the throwing in of extra veins is probably caused by the necessity for greater strengthening of the wings. Just how these extra veins arose is not easy to explain. They may be sports, or reversions to an ancestral type, or the result of extra growth-energy or caused by the splitting of normal veins so that it is rather difficult to say just what factors bring about this extra amount of variation. While we speak of these as abnormal veins it must be noted that we do not know whether they are really abnormal or whether they are but the manifestations of a tendency possessed by all bees but which can develop only under certain conditions, just as the ovaries of the workers can develop only when extra room and food are provided.

If then germinal variation will not explain all these variations and if we accept the explanation offered as a partial and possible statement of the cause, then it would appear that the mere chance as to which cell happens to be the receptacle of a drone egg determines its variation. While it is probable that even this "chance" is according to fixed law, the fact remains that in any event this law is beyond the possibility of formulation from any observations except those extending over far more individuals than those here used. On this account we consider ourselves justified in our tabulation of results rather than in the plotting of curves and expression in mathematical formulæ, since that would be undesirable except with far more measurements and with material gathered under conditions better controlled. Our tables show the variation as it actually exists in a state of nature and the real laws can be worked out only from observations from control experiments and this it is hoped will be possible in the near future.

We do not wish to be considered as advocating the inadequacy of the theory of germinal variation to explain variation, since we have no means of knowing whether these variations can be inherited but simply wish to express the facts as we find them, and leave the explanation of the bearing of germinal variation on this problem for future investigation.

October 1, 1903.

Explanations of Figures.

Fig. 1. Fore wing of honey bee, normal. Cells and veins are named according to Comstock and Needham.

Fig. 2. Hind wing of honey bee, normal.

Fig. 3. Typical hymenopterous wing according to Comstock and Needham.

Fig. 4. Part of fore wing of honey bee showing (in dotted lines) where accessory veins were seen to occur in the wings examined. Lettering purely arbitrary as explained in text.

THE OVARIAN STRUCTURES OF THE VIVIPAROUS BLIND FISHES, LUCIFUGA AND STYGICOLA.[1]

HENRY H. LANE, A.M.

I. Introductory.

During the spring of 1902, Dr. C. H. Eigenmann collected a number of specimens of blind fishes in the caves of western Cuba, within a radius of 130 kilometers of Havana. The fishes belong to the two distinct but closely related genera, *Lucifuga* and *Stygicola*. It has been my good fortune to have the opportunity of studying the reproductive organs — more particularly, the ovarian structures — of these blind fishes, with special reference to their method of reproduction. It was discovered upon examination of the specimens that they are viviparous,[2] a fact long known in regard to some of their deep-sea relatives. Owing to the lateness of the season when they were collected, unfortunately but one female was pregnant. This one measured only 65 mm. in length and contained four fœtuses — borrowing a term to designate the post-larval stages of the young until birth — 18–20 mm. long. These fœtuses were in an advanced stage of development, very probably being within a few days, or possibly hours, of birth, since a number of young only 25 mm. long were caught in the water. No other prenatal specimens having been secured, it has been impossible to study the early stages of development. My attention has been particularly directed to the ovarian structures of the mature females secured. A few of the young specimens, evidently taken not long after birth, were also examined.

I wish here to express my deep sense of obligation to Dr. C. H. Eigenmann for his assistance and criticism in the preparation of this paper.

[1] Contributions from the Zoölogical Laboratory of Indiana University, No. 58.

[2] Eigenmann, '03, p. 236, pl. 21.

II. Systematic Position of Lucifuga and Stygicola.

Poey[1] ('60) described these fishes in 1860, giving them the generic name of *Lucifuga*, recognizing them, however, as two species, *dentatus* and *subterraneus*. Later Gill, '63, separated *dentatus* from the other and created the genus *Stygicola* for it. The two genera are different in that *Stygicola* has teeth on the palatines, where *Lucifuga* has none, and the teeth in the jaws of the former are larger than those of the latter. There is also a very noticeable difference in the depth of the head at the nape, in adult individuals. The two species or genera are however so nearly alike that it is only after a prolonged comparison that the above technical differences were made out.

The several species of blind cave fishes, found in Indiana, Illinois, Kentucky and Missouri, are not related to the Cuban species. The latter are descended from marine forms which have worked their way through underground channels into the Cuban caves. Related genera that still live in the ocean about Cuba are *Brotula*[2] and *Ogilbia*[3] in moderate depths and *Bassozetus*[4] and *Aphyonus*[5] in deep water.

All of these genera belong to the family Brotulidæ, a deep-sea group comprising about forty-five genera and one hundred species, living mostly in the tropical seas of both hemispheres. The two genera under consideration in this paper are the only ones found in fresh water. Jordan and Evermann (*op. cit.*, Pt. III., p. 2498) observe very properly that "these fishes are closely related to the Zoarcidæ. In spite of various external resemblances to the Gadidæ their affinities are rather with the blennioid forms than with the latter."

III. Historical.

Numerous contributions to our knowledge of viviparity in fishes have been made from the time of Cuvier to the present.

[1] *Lucifuga*, Poey, "Memorias," II., 95, 1860 (*subterraneus*); *Lucifuga subterraneus*, Poey, "Memorias," II., 96, 1860; *Lucifuga dentatus*, Poey, "Memorias," II., 102, 1860; *Stygicola dentata*, Gill, *Proc. Ac. Nat. Sci. Phil.*, 252, 1863.

[2] *Brotula* (*vid.* Bull. 47, U. S. Nat. Mus., Jordan & Evermann, "Fishes of North America," Pt. III., p. 2500).

[3] *Ogilbia* (*vid. idem*, Pt. III., pp. 2502, 2503).

[4] *Bassozetus*, Gill (*vid. idem*, Pt. III., p. 2507).

[5] *Aphyonus*, Günther (*vid. idem*, Pt. III., p. 2525).

Among the most important ones are the following, most of which I have consulted in connection with my own investigation :

Cuvier and Valenciennes, in their " Histoire Naturelle des Poissons," I., Paris, 1828, have a short general account of viviparity in fishes, and mention is made of it frequently throughout their work in the description of such fishes as bring forth living young. Much of their work has been superseded by the more accurate observations of later investigators.

Rathké, in 1833, published his " Bildungs- und Entwickelungsgeschichte des Blennius viviparus odes des Schleimfisches." This was long the best paper on the subject.

In 1844 (*Ann. des Sc. Nat.*, t. I., 3d series, p. 313) Duvernoy published a paper on *Pœcilia surinamensis*, which is frequently referred to by more recent writers, but which I have not had the opportunity of consulting.

In 1846, Cuvier and Valenciennes described the genus *Anableps*, one species of which, *A. gronovii*, formed the subject matter of an important paper by Jeffries Wyman, in the *Boston Journal of Natural History*, Vol. VI., No. IV., p. 432, 1857. I shall refer to this article more at length below.

In 1853, Louis Agassiz (*Am. Jour. of Science*, XVI., 2d series, Nov., 1853) described a new family of fishes from California — the Embiotocidæ, which embraces the genus *Cymatogaster*. The only species of this genus, *C. aggregatus*, was studied in detail by Dr. Eigenmann, and the results published in the *Bulletin of the U. S. Fish Commission*, Vol. 12, p. 401, 1892 (1894).

Another very important paper, " On the Development of Viviparous Osseous Fishes and the Atlantic Salmon," by John A. Ryder (*Proc. U. S. Nat. Mus.*, 1885, pp. 128–162, Pls. VI.–XII.) will be noticed frequently below.

In 1887, Dr. Franz Stuhlmann made a detailed study of *Zoarces viviparus*, Cuv., the results of which he published under the title, " Zur Kenntnis des Ovariums der Aalmutter (*Zoarces viviparus*, Cuv.)." Frequent references to this volume will be made below.

IV. Viviparity in General.

Cuvier and Valenciennes (*loc. cit.*) give a general account of viviparity in fishes so far as known at that time ; but since their

statements are now either matters of common knowledge or else not in accordance with the facts as revealed by later investigations, I shall not speak of their work further.

Wyman (*op. cit.*) classifies viviparous fishes into two groups "according to the position occupied by the embryo during the period of growth. In the first group may be arranged those fishes in which *the ovum leaves the ovary in an undeveloped state, and in which the process of colution* (*sic*) *is not commenced until it reaches the lower portion of the oviduct.* The fishes which this group comprises are nearly all, if not all, Plagiostomes. The best known are *Spinax*, *Carcharias*, *Mustellus*, *Galeus*, and *Torpedo*. . . . II. In the second group those fishes are comprised in which *the gestation is wholly or in part ovarian*, the last stages only of the process usually occurring in the oviduct. Among the genera included in this division are *Silurus*, *Blennius*, *Anableps*, *Pœcilia* and *Embiotoca*. In all of these genera impregnation takes place in the ovary, and, as seems probable, while the ovum is still invested with its original envelopes."

Wyman found that each of the fœtuses in *A. gronovii* is enveloped in a separate sac of vascular tissue, much too large for the fœtus enclosed, the extra space being filled up with an albuminous fluid. He seems to regard these fœtal sacs simply as extensions of those within which the ova were suspended.

Eigenmann, considering only teleosts, found two types of viviparity (*op. cit.*, p. 404); he says:

"At least two types of viviparity may be distinguished in fishes; first, those in which the yolk furnishes all the intra-ovarian food; and second, those in which the greater part of the food is furnished by the ovary.

"In the first type the number of young is not less than in related oviparous forms, while the number of young in the second is always greatly reduced. . . . The size and development of the young in this class (type I.) of fishes at the time of birth is of course much less than in the second class of viviparous fishes."

As will be seen, *Lucifuga* and *Stygicola* belong to the second type of both Wyman and Eigenmann. The number of young is small and they are born in quite a mature condition.

V. Gross Anatomy.

For the sake of clearness the following terms will be used in the sense here given :

Oviduct — the single duct leading from the ovary to the urogenital pore. Ovisac — the forward continuation of the oviduct which covers the ovary. Ovary — the structure containing the eggs. Stroma — the supporting tissues of the ovary itself.

The term ovary is also used in a general way to include the ovisac and the ovarian structures proper. The context in every case will determine what is meant.

In *Lucifuga* and *Stygicola* the ovary is enclosed between two layers of peritoneum above the posterior portion of the alimentary canal. It may extend so far forward as to lie in part even beside the stomach.

The ovary has a bilateral arrangement. Externally it is a Y-shaped, bifurcated, subcylindrical organ, whose greatest diameter is immediately posterior to the point at which the division begins (Fig. 1). The two horns lie on the right and left sides respectively and may enclose between them the posterior portion of the stomach. Interiorly the stem of the Y is divided by a median partition with which the ovarian structures proper are associated and which extends to near the oviduct, though here only the portion attached to the ventral wall is found (Fig. 2). From the tips of the ovarian horns slender though comparatively strong threads of connective tissue, inclosing blood vessels, run forward and fasten to the peritoneal walls, thus very securely holding the ovary in position. Dorsally, the ovary is attached to the peritoneal lining of the body cavity by the mesovarium ; ventrally, there is a corresponding attachment, the mesorectum. The oviduct, which opens externally at the urogenital pore, increases gradually in size as it approaches the ovary and finally merges into the ovisac, or outer wall of the ovary.

A somewhat immature specimen shows finely those structures connected with the support of the ovary. In it one sees that each horn is supported by its own fold or lamina of peritoneum ; that these two laminæ become united at or near the point of division of the horns and are continued posteriorly as a single though thicker mesovarium supporting the body of the ovary

and the oviduct. Below there is no sign of a mesorectum in the region of the ovarian horns, except for a short distance near the base of one of them, but posterior to them there is such a membrane, inclosing several blood vessels, and itself somewhat thicker than the mesovarium above. It is also clear from an examination of this ovary that, as will be noted more particularly below, the egg-bearing tissue, the ovary proper, forms a thick median partition in the ovisac.

The external appearance of the ovary agrees very closely with Eigenmann's description (*op. cit.*, p. 418) of that of *Cymatogaster*:

"The ovary is a spindle-shaped bag, divided anteriorly into two arms which indicate the bilateral origin of the present structure. One of these arms, the left, is usually smaller than the other. . . . The ovaries of the two sides have evidently been united from behind forward, so that externally only the two anterior horns show the bilateral structure, and one of these horns seems to be in process of phylogenetic resorption."

While there is frequently a difference in the size of the two horns in *Lucifuga* and *Stygicola*, there is no uniformity in this matter. There seems to be no evidence that the right or left portion of the ovary is "in process of phylogenetic resorption." Ryder found that in *Gambusia patruelis* "the ovary is a simple unpaired organ, the greater part of which lies on the right side of the body-cavity below the air bladder. . . ."

The size of the ovary varies, of course, with the age and size of the female, as well as with the state of development of the ova or embryos contained in it. One female of the genus *Lucifuga*, which had a length of 65 mm. and which contained four fœtuses nearly ready to be born, had an ovary with a length — measuring to the extremity of the longer horn — of 16 mm., and a diameter of 8 by 9 mm. As the fœtuses were 18 to 20 mm. long, it was not surprising to find that their tails were bent over. Another female of the same genus, 83 mm. long, had evidently given birth to young only a short time before her capture and had an ovary 12 mm. in length.

The point of division into the two horns is usually about five twelfths the distance from the anterior tip of the ovary to its posterior end. As already stated, the two horns rarely show equal

PLATE 1

Fig 1

Fig 4

Fig 2

Fig 5

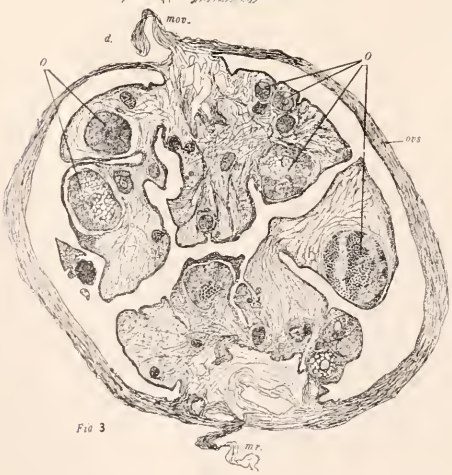

Fig 3

Fig 6

development, there being always a more or less marked difference in size.

Between the ovisac and the ovary proper there is a lumen of varying size. When there are larvæ present, the ovisac and the oviduct are extremely thin and so stretched, especially near the close of gestation, that their cellular structure cannot be made out with any satisfaction (Fig. 5). Shortly after birth of the young they contract and assume the form and appearance found in the ovaries of mature but non-pregnant females (Fig. 3). The wall of the ovisac is then quite thick, and the lumen very small. The histological structure of the ovisac will be described below.

In non-pregnant ovaries, the stroma is a mass, which, internally, has a bilateral arrangement and occupies most of the space within the ovisac (Figs. 2, 3). It is, in general shape, fusiform with its largest diameter just posterior to the division of the ovisac into the two horns down both of which it is continued along their median surfaces, forming the prongs of a Y. In the middle of the ovary the stroma forms a median partition; somewhat posteriorly this partition is cut across (Fig. 3), and still further back only the ventral part remains (Fig. 2). It has many lobes which are usually somewhat pointed and comparatively large and distinct, the indentations sometimes leaving merely a "neck" of tissue to support them. The whole stroma at this time is fully distended by the large amount of lymph contained in the sinuses described below. Where the ova are well advanced they can be seen by the unaided eye in the form of opaque dots. When the ova are surrounded by follicles, they lie some distance below the surface of the stroma and there is a tubular indentation of the epithelial covering of the latter down to the follicle (Fig. 7, *D*). In a circular space over the egg the epithelium is apparently continuous with the follicle. It is only on very close inspection that the independence of the follicle can be made out. It is then found to be of only a single cell in thickness beneath the epithelial indentation. A similar position of the epithelium was noted by Stuhlmann over the ova in the ovary of *Zoarces* and was called "Delle" by him.

The pregnant ovary is quite different in appearance from that of a non-pregnant female. A cross-section of the former shows

it to be an almost bilaterally symmetrical organ, but without any folds or pockets in which the embryos are contained as is the case in *Cymatogaster*[1] and numerous other species of Embiotocidæ. During the intra-ovarian development of the embryos, or rather the development of the fœtuses within the oviduct of *Lucifuga*, the ovarian structure proper or stroma which forms the thick median partition in non-pregnant ovaries, becomes gradually reduced and compressed into a narrow wall (Fig. 5). The stroma is much thickened both dorsally and ventrally near the oviduct (Fig. 4), where the partition is incomplete, but anteriorly its greatest thickness is near the median plane (Fig. 5). The arrangement of the stroma in each horn of the ovary is as in non-pregnant ovaries (Fig. 6, *ov.st.*).

The single oviduct runs from the caudal end of the ovary proper to open at the urogenital pore. In pregnant females it is widely distended for some distance when the fœtuses are well advanced, but in the non-pregnant females it is a rather cylindrical, thick-walled, muscular tube with numerous folds or laminæ on its inner surface, covered with a layer of columnar epithelial cells, 12 μ in depth. It is not materially different, except as to dimensions, from the ovisac described above. Stuhlmann[2] similarly found the oviduct of *Zoarces* to be a tube composed of the same cell-layers as the ovary, with the exception of the "germinal" and follicular epithelia.

VI. Histological Part.

I. The Walls of the Ovary or Ovisac.

The following system will be used to facilitate cross references to the descriptions of the various ovaries. Each ovary will be referred to by a letter, *A*, *B*, *C*, etc., the meanings of which are as follows:

A represents a female of the genus *Stygicola* with a length of 95 mm.

B represents a female of the same genus, but with a length of 128 mm.

C represents a female of the genus *Lucifuga*, length 87 mm.

[1] Eigenmann, *op. cit.*, p. 418.

[2] *Op. cit.*, p. 10.

D and *E* represent females of the same genus with a length of 83 and 65 mm. respectively. *E* was pregnant; the others were non-pregnant.

An examination of the ovisac and oviduct reveals quite a range of variation, depending in the main upon the condition of pregnancy or upon the length of time that had elapsed since the close of that period.

In the ovary of *D*, which had not been pregnant at all, or at least for so long a time that the ovarian structures had regained their normal form, the wall of the oviduct and the ovisac is from 100 to 150 μ in thickness at different places. Structurally, the ovisac consists of at least four cell-layers. The outer, a sinuated, peritoneal layer, immediatly beneath which there is a thicker layer of longitudinal muscle fibers; below this there is another somewhat thicker transverse band of muscle fibers; on the inner surface there is an epithelial layer *containing numerous capillaries.* This will be described in detail below. The nuclei of the longitudinal band are rod-like in appearance; the nuclei of the second muscle layer appear more nearly round, being evidently the cross-sections of nuclei of the same form as those in the longitudinal band. The innermost layer of epithelial cells has nuclei oval or round in shape, while the peritoneal layer shows few nuclei, but those which do appear are rod-shaped in section. Quite numerous capillaries are found between the cells of these several layers and in some places there are large blood vessels.

In the ovary *A* from a female which had evidently given birth to young but a short time previous to her capture, the ovisac measures only 15 to 20 μ in thickness. Structurally it consists of four or five thin cell-layers, between which there are anastomosing capillaries. The outermost layer consists of peritoneum, the cells of which are very much elongated and compressed. The muscle fibers beneath are mostly transverse and of the non-striated type. The inner layer is epithelial and is also much compressed. The nuclei of the muscle-fibers are long, narrow, rod-like structures which stain deeply, as would be expected, with hæmatoxylin: the nuclei of the epithelium are oval in form. The condition of this ovary does not permit me to go into greater detail.

PLATE II

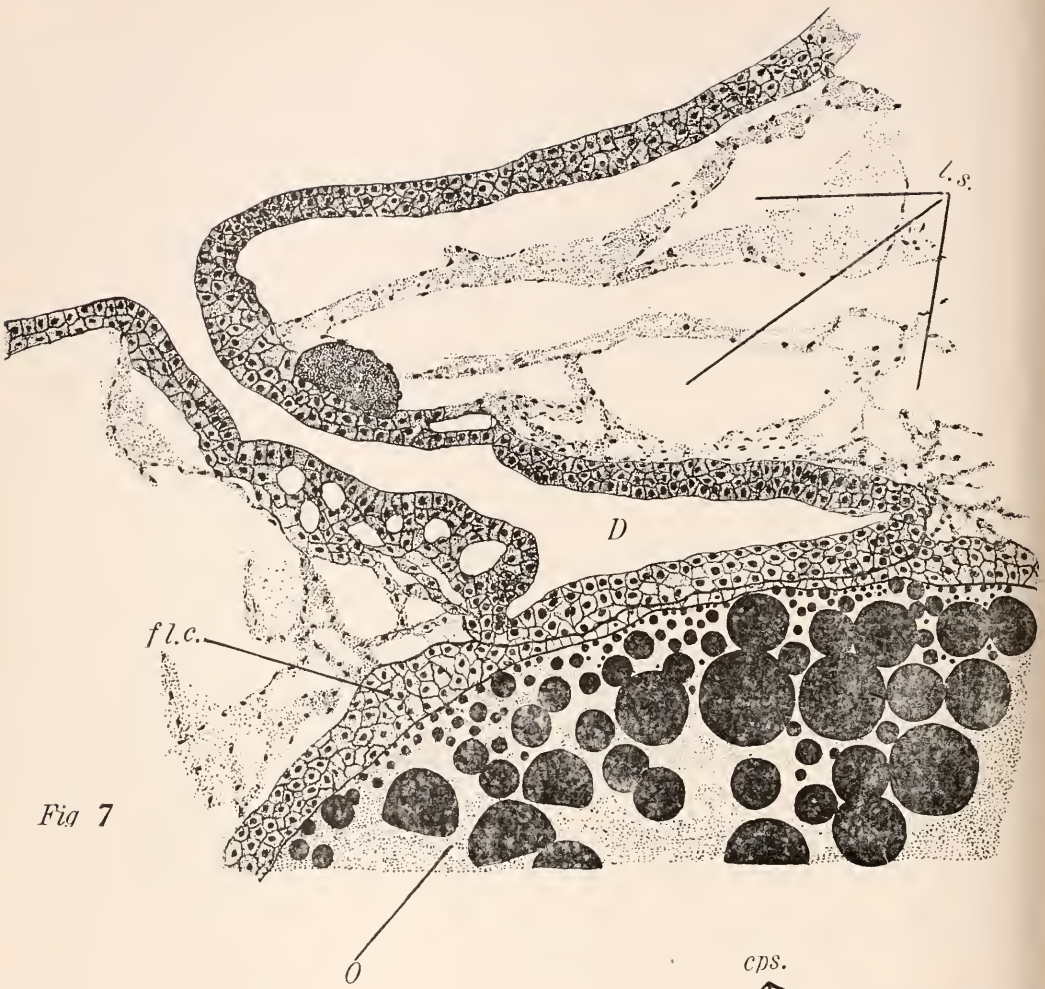

Fig 7

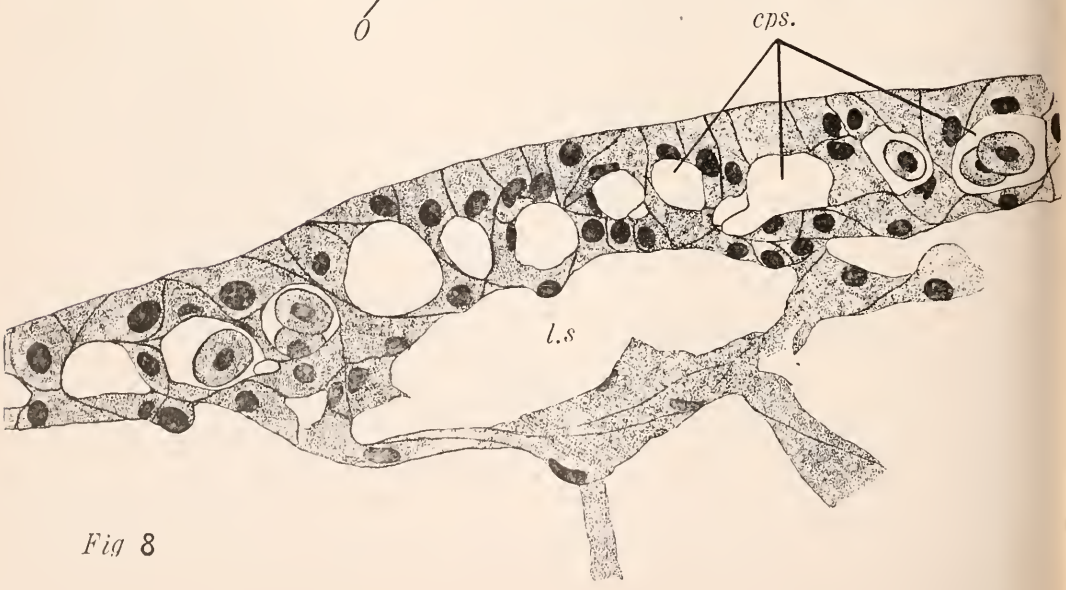

Fig 8

In the ovary of the pregnant female *E*, whose young were almost ready for birth, the ovisac is thinner than in the case just described. The different cell-layers can scarcely be distinguished, though where the cells themselves are visible, the nuclei in section have the rod-like form mentioned above. The capillaries have mostly disappeared, apparently being closed by the crowding together and stretching of the cellular structure ; but in places one comparatively large vessel appears, containing two or more rows of corpuscles, side by side. At this place the wall is enlarged somewhat to accommodate the vessel.

In the ovary of *C*, which contained ova quite well advanced, the ovisac is very similar to that described for *D*. But in some portions of this ovary the muscle layer is restricted almost entirely to longitudinal fibers, the transverse layer being much reduced. Capillaries penetrate freely through these muscle layers in all directions ; those in the lining epithelium are larger than those in the other specimens already described.

In the ovary of *B* the ovisac is similar to that in *C*, but the peritoneal covering is not so distinct ; there is the same arrangement of muscle-fibers — the outer longitudinal and the inner transverse, the latter being much the deeper. The innermost epithelial layer is composed of " pavement " cells with quite large distinct nuclei. Numerous capillaries are found in this inner lining.

Compare in this connection Eigenmann's description of the ovarian wall in *Cymatogaster* (*op. cit.*, p. 418) :

" The ovarian walls are composed, first, of the thin peritoneal membrane ; second, of a layer of longitudinal muscle fibers ; third, of a layer of circular muscle fibers, inside of which there is, in places, a layer of longitudinal fibers ; fourth, of a very thin layer of cells with flattened, deeply stainable nuclei ; fifth, of a layer of epithelium. This layer is derived from the peritoneum."

Stuhlmann found the ovarian wall in *Zoarces* to have a tolerably deep, non-striated, muscle layer, the fibers of which were closely packed together next to the peritoneal covering, but toward the lumen they were split apart by numerous sinuses containing blood vessels. The oviduct was similarly composed, except that there were few if any clefts between the fibers and there were fewer blood vessels.

The unique feature in the ovisac as well as in the epithelial covering of the stroma of *Lucifuga* and *Stygicola* is the presence of capillaries in the lining epithelium (Fig. 8). So far as could be determined this condition has never been observed in the ovary of any other form. So numerous are these capillaries that they attract attention at the first glance. The epithelium itself is often reduced to extreme thinness, sometimes serving merely as a membrane to contain the blood.

VII. The Ovary.

The ovarian structure itself is highly vascular and much lobed. There is a tendency in some instances for these lobes to be arranged in a bilaterally symmetrical pattern, when seen in cross-section, though this is not equally evident in all ovaries or even in all parts of the same ovary. The ovarian structures of the different specimens examined, while presenting numerous points in common, are yet characteristically different in every case.

The ovisac of *A* had but recently contained young, to judge from its extreme thinness ; the stroma was so large that it gave promise of containing embryos. Instead of that condition, however, it was found that the large size was due to the mass of stroma which is composed in part of highly vascular tissue. Numerous blood vessels penetrate the stroma in all directions — while around the ova themselves there is a network of capillaries. The greater portion of the stroma is split up into numerous sinuses, many of which are larger than any of its blood vessels. These are closely similar in appearance to the " lymph-spaces " described by Stuhlmann (*op. cit.*, p. 19) for *Zoarces* and no doubt serve the same purpose (Fig. 7, *l.s.*).

The ova of *A* are few in number, less than ten over 60 μ in diameter appearing in any cross-section. Five or six ova are of quite large size, visible even to the naked eye, and measuring from 300 to 800 μ in diameter. They have a large amount, proportionately of yolk-substance. The smaller ova are about 50 to 60 μ in diameter, and are of the usual appearance of ova of that size. The cells of the stroma in this ovary are very irregular in shape, indistinct in outline, and usually of inconsiderable size. The nuclei are round, oval, or elongated, appar-

ently influenced as to form and shape by the cell-body. The entire surface of the stroma is covered by a layer of epithelium, with a depth of 10 to 15 μ. The nuclei of these epithelial cells appear quite distinct, are of a comparatively large size, and are round or oval in shape.

By far the largest amount of space in this epithelial layer is given up to the numerous capillaries contained in it. They are so numerous that, in cross-section, they appear as a row of large perforations, there being no more than a scant cell thickness between them. The average diameter of these capillaries is less than eight micra, in many instances being only five micra. This condition is comparable to that described above for the ovisac and is also unique (Fig. 8).

The ovarian stroma of *E*, which contained the mature fœtuses, has been squeezed and crowded into a median position by the young (Fig. 5). The cellular structure resembles that of the ovary just described, except for such variations as would be caused by its closely packed condition. The capillaries of the epithelial layer, covering its surface, are not so numerous as in the ovary of *A*, but are of larger size. The larger blood vessels are more nearly cylindrical in form and have their walls more thickened than have those in the first ovary. The lymph-spaces in this ovary are compressed by the fœtuses and temporarily eliminated.

Quite different in appearence from either of the two just described, though somewhat intermediate between them in some respects, and more advanced than the first in others, is the ovary of *D*. In this the ovarian stroma has not so many nor such large lymph-sinuses as *A*, but on the contrary has more nearly the appearance of that in *E*, from which it differs conspicuously, however, in not showing a "crowded" appearance, and in having quite numerous ova of various sizes, though none of the latter are so large as those of *A*, and in many cases are grouped together in "nests" in a way largely unknown in *A*. The blood vessels are comparatively numerous, large and quite thick walled. The capillaries in the epithelial covering of the ovary — so conspicuous is that of *A* — are so few in this case as to be visible only when carefully searched for. The cellular structure near and

next to the surface is dense and without important sinuses. The outlines of the cells are very indistinct, but the nuclei are altogether similar to those of the ovaries previously described.

The ovary of *C* approaches more nearly to the condition of *A* than has any of the others; but it differs very characteristically, since in many places it contains "nests" of ova much more conspicuous than any seen in *A*, while the largest ova in this specimen are larger than those in *A*. The ovarian structure itself, while evidently of the same character as that of *A*, does not contain quite so many lymph-spaces, and the walls of the sinuses are somewhat thicker and denser.

The ovary of *B* is almost exactly in the same stage as that of *A*. It differs from *C* in that the egg-nests have given place to single ova of considerable size and greater development than most of those in the latter.

It will be noted that the "nests" of ova are conspicuous in *Lucifuga*, though inconspicuous or lacking in the specimens of *Stygicola* examined. Whether this is a constant distinction can only be determined by the examination of more material than I have in hand.

VIII. Blood Supply to the Ovary.

A small artery, with a diameter, in different ovaries, of 20 to 75 μ, enters each horn of the ovary and runs back near the inner surface of the horn. In the main portion or body of the ovary, the two arteries occupy parallel courses near the center, separated by perhaps one third the diameter of the ovary. Since none of the specimens at hand were injected, the course of these arteries could not be traced except in a general way. But it is plain that they extend posteriorly in a tortuous course through the ovary and give off numerous branches, which find their way to or toward the surface, where they form the capillaries so distinctly visible in some of the ovaries in the epithelial covering. The blood from the epithelial capillaries of the anterior half of the ovary is collected by veinlets, frequently quite large and distinct in the vicinity of the larger ova, which join to form larger veins that pour their contents into the chief vein of the ovary at the "horseshoe bend" (infra). This largest vein has two branches

(one going out by either horn) which are united near the point of division of the ovarian horns, forming a single "horseshoe"-shaped vessel. The veinlets which return the blood from the posterior part of the ovary collect into one vessel which joins the right horn of the "horseshoe" at a considerable distance in front of the fork of the ovary, after running above and parallel to the portion with which it unites, for the distance, in one specimen at least, of nearly 2 mm.

It quite frequently occurs that red-blood corpuscles are present in the ovarian sinuses of *Stygicola* and *Lucifuga*, though their presence may be due to accident. As indicated elsewhere, these sinuses are very probably filled with a plasma or lymph.

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Explanation of Plates.

Fig. 1. External ventral view of the ovary of *Stygicola*. Portions of the peritoneal covering are visible along the sides.

Fig. 2. Cross-section of ovary near the beginning of the oviduct proper. Two large ova at the sides.

Fig. 3. Cross-section of non-pregnant ovary with stroma in two lobes — one dorsal, the other ventral.

Fig. 4. Cross-section of pregnant ovary. The section is made through a region corresponding to that of Fig. 3.

Fig. 5. Cross-section of pregnant ovary through the middle portion. The ovisac collapsed when the fœtuses were removed.

Fig. 6. Cross-section of pregnant ovary through the horns.

Fig. 7. A portion of a cross-section of a non-pregnant ovary, showing a part of a large ovum (*o*) surrounded by its follicle (*fl.c.*); the epithelial covering of the stroma dips down, forming a tube to the ovum (*D*). Bausch and Lomb one sixth objective; 2-in. ocular; tube length, 160 mm.

Fig. 8. Portion of the epithelial covering of a non-pregnant ovary showing the capillaries (*cps.*). Bausch and Lomb one twelfth objective; 1-in. ocular.

a. anterior.
cps. capillaries.
d. dorsal.
D. the "Delle."
fl.c. follicular cells.
l.s. lymph sinus.
mov. mesovarium.
mr. mesorectum.
o. ovum.
ovs. ovisac.
ov.st. ovarian stroma.
p. posterior.
v. ventral.

All drawings by the author; outlines made with Abbé camera; details put in free-hand but with the closest possible regard to accuracy.

Vol. VI. *January, 1904.* *No. 2*

BIOLOGICAL BULLETIN.

FORM-REGULATION IN CERIANTHUS, III.

The Initiation of Regeneration.

C. M. CHILD.

In the first paper of this series ('03*a*) the typical course of regeneration in a cylindrical piece was described; in the second paper ('03*b*) some of the factors influencing the process of regeneration as a whole were discussed; these papers have served to clear the ground for a detailed analytical study of the process of regeneration in *Cerianthus* in its various manifestations. In this and following papers of the series various phases of this subject will be considered.

Changes in Form Consequent upon Section.

The reduction in size of the opening at the end of a cut piece by the bending inward of the cut margins was described in its simplest form in the first paper of this series. A somewhat more general consideration of this peculiar process is necessary before proceeding to the discussion of other points.

Early in the course of my experiments upon *Cerianthus* it was noted that in nearly every case, however the pieces might be cut, the body-wall became rolled or folded in such a manner that the opening into the enteric cavity resulting from the cut was much reduced in size or was closed by approximation or contact between different parts of the body-wall. The usual result of the infolding is the complete removal of the entodermal surfaces from contact with the external water, *i. e.*, the piece rolls up or closes in such manner that the entoderm is on the inside. For convenience we may designate inrolling about a transverse axis as transverse inrolling, and inrolling about a longitudinal axis as longitudinal inrolling.

At first glance this process appears much like an adaptive reaction. In some cases it is almost as if the animal or part were

consciously closing the artificial openings. In the following paragraphs the principal forms of inrolling in the cut pieces are described.

The case of the closure of the ends of a cylindrical piece which was described in the first paper is the simplest of all. Collapse occurs with the escape of the water from the enteron and within

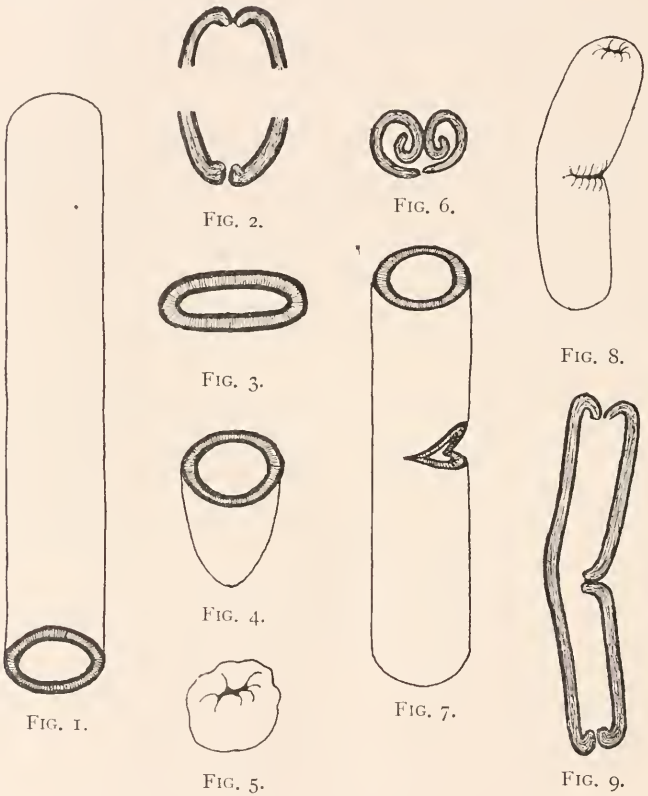

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

a few moments the cut ends begin to bend inward and finally close the openings except for the small slits between the folds. The diagrams, Figs. 1,[1] 2 and 3, illustrate this case, Fig. 1 representing the cylindrical piece at the time of section, Fig. 2 the longitudinal section of the ends after the bending in of the cut margins, and Fig. 3 a transverse section, indicating the flattening

[1] The diagrams representing the inrolling are much less highly magnified than preceding figures.

of the piece as it lies on a flat surface. In pieces of this kind complete collapse and contact of the body-walls is prevented by the large mass of mesenteries and mesenterial filaments which occupy the enteron. These are not represented in the figures, but they fill the whole enteron after collapse. Any solid mass in the enteron would of course have the same effect.

A piece cut from the extreme aboral end of the body (Fig. 4) differs in certain respects from the piece just described. Figs. 5 and 6 show the changes in a piece of this kind. Here the cut end becomes rolled inward to a much greater extent than in the previous case so that the enteron is nearly filled by the inrolled portion and the cut surface is so situated that closure by growth of new tissue from this surface is impossible. The reason for the greater degree of rolling in this piece as compared with the longer piece is undoubtedly to be found in the absence of mesenteries, except a single pair, in the aboral region. Since the enteric cavity is not filled with a mass of mesenterial filaments as in a region further orally the inrolling continues until the entire cavity is practically obliterated by the inrolled parts.

If a cut be made in one side of the body or a piece removed as in Fig. 7, the cut edges roll inward as in other cases, but in addition to this the body becomes bent at the level of the cut, so that here also the inrolled edges are brought into contact (Figs. 8 and 9).

The widest departures from the typical form are found, however, in those pieces which were cut longitudinally as well as transversely. In these the results differ to some extent according to the shape and relations of the pieces. Fig. 10 represents a cylindrical piece split longitudinally on one side. One form after collapse and inrolling of cut margins is shown in Fig. 11. Fig. 12 represents a transverse section and Fig. 13 a longitudinal section of one end. In Fig. 14 another form of closure is represented; here the ends fold over to a greater extent so that the opening is entirely on one side of the piece. Fig. 15 represents a transverse section of this piece and Fig. 16 a longitudinal section in the plane indicated by the vertical line in Fig. 14.

In most cases, however, the right and left longitudinal cut edges do not roll inward with equal rapidity and the result is

that the piece rolls up spirally on its longitudinal axis. Such a case is shown in Fig. 17; here the inner and outer coils of the spiral are on the same level at the outer end of the piece. Fig. 19 shows a spirally coiled piece in which the inner coils are higher than the outer. Figs. 18 and 20 represent longitudinal sections of one end of these pieces and Fig. 21 a transverse section.

The Figs. 11–21 represent only the chief types resulting from pieces like Fig. 10. All possible intermediate forms and modifi-

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

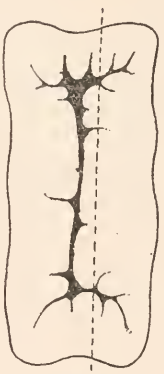

Fig. 14.

Fig. 15.

Fig. 16.

cations of these different types occur, the differences depending on various conditions, but chiefly on the relative rapidity of the inrolling in the different directions.

Semi-cylindrical pieces or longitudinal strips may roll either longitudinally or transversely. The greater the length and the less the breadth of the strip the more likely it is to roll transversely. Figs. 22–24 show a strip and two forms of transverse rolling which it may undergo. The longitudinal strips

often roll longitudinally soon after section, but by gradual inrolling of the ends finally become rolled transversely.

Loeb ('91) has suggested that the cut edges roll inward because the inner layers of the body-wall are stretched to a greater degree than the outer layers; this view assumes that all layers are more or less similar in elasticity and therefore that the layer that is most stretched will undergo the greatest contraction when the tension ceases. It is difficult to understand why one layer of the body should be more stretched than another, since all have been subjected to the same conditions, viz., the tension resulting from the fluid pressure on the walls of the enteron.

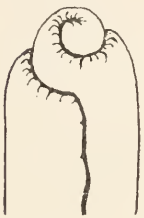

FIG. 17.

FIG. 18.

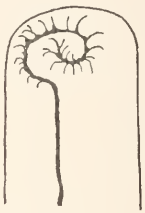

FIG. 19.

FIG. 20.

FIG. 21.

FIG. 22.

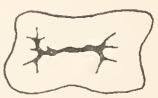

FIG. 23.

FIG. 24.

I am inclined to believe that this remarkable capacity for rolling which the pieces exhibit is due primarily to a difference in elasticity between the different layers of the body-wall, though it may be increased or modified by other factors. The succession of layers in the body-wall is as follows: ectoderm, longitudinal muscles, mesoglœa, entoderm. The mesoglœal layer is fibrillar in appearance and, while not as thick as the muscular layer, is well developed.

Judging from the fact that this layer is not folded or wrinkled, even in strongly contracted animals, the inference that it possesses

a considerable degree of elasticity appears justifiable. Under normal conditions the body-wall is subjected to tension. By section of the body at any point the internal pressure is removed and collapse occurs; the body-wall is no longer under tension and contraction of the elastic layer begins. If the ectoderm and muscles are to a large extent passive in this elastic contraction the result will be not simply a reduction in surface area, but an inrolling of the body-wall, since the mesoglœa is situated near its inner surface.

The fact that the region near the cut surface is always more strongly rolled than other parts may perhaps be the result of the direct injury to the tissues in this region, causing contraction, but here as elsewhere the contraction must be greater in the inner portions of the body-wall than in the outer, otherwise inrolling could not occur. More probably, however, the greater degree of inrolling near the cut surface is largely, if not wholly due to the fact that the physical obstacles to the inrolling offered by resistance of other tissues, etc., are much less near a free end or cut surface than elsewhere and the effect of elasticity is therefore greater. It appears probable from the preceding considerations that the mesoglœa plays the chief part in the inrolling about the cut surface as well as in regions distant from it.

Objection to this view may, however, be made on the ground that a tonic muscular contraction resulting from the injury is not only a possible but a much more probable cause of the inrolling. A brief consideration of the facts is sufficient to show that the inrolling cannot be explained as the result of muscular contraction.

This is evident first from the fact that it occurs in all directions, longitudinally and obliquely as well as transversely. If it were the result of muscular contraction we should expect it to occur only transversely since the body-wall contains only longitudinal muscles. It is difficult to understand how the contraction of longitudinal muscles could account for the inrolling of a longitudinal cut margin, since the muscle fibers are parallel to the cut. Moreover, there is no apparent reason why the inner portions of the muscular layer should contract more strongly than the outer, since all must be equally affected by the injury. And finally, observation renders it very evident that the inrolling is

not due to muscular contraction, for although strong contraction occurs at the time of section the muscles relax within a few moments and before inrolling begins. Moreover, the muscular contraction consequent upon stimulation of pieces after inrolling has occurred causes in most cases a more or less complete unrolling, provided the inrolling was in the transverse direction but does not affect inrolling in the longitudinal direction. In the light of these facts it is difficult to escape the conclusion that the inrolling is caused by some part of the body-wall axial to the muscular layer, viz., either entoderm or mesoglœa. The delicate cellular layer of entoderm cannot be supposed to possess any such elasticity; there remains therefore only the mesoglœa.

It now remains to consider whether the different forms of inrolling described and figured above are all explicable on the basis of elasticity of the mesoglœa. For this purpose we may regard the tension as resolved into longitudinal and transverse components.

As regards the inrolling of cylindrical pieces with transverse cut margins (cf. No. 1 of these studies, '03*a*, also Figs. 1 and 2 of the present paper), it is easy to see that it can proceed only a certain distance. Since the elastic tension is present in all parts of the cylindrical piece reduction in size may occur, but the cut ends are the only regions where marked change of form can take place. These are bent inward until the more prominent folds come into contact and the size of the opening is reduced. Beyond this the inrolling cannot go since contact between different parts of the margin and the radial folds into which the contracting margin is thrown both oppose further change. The appearance of the radial folds requires a word of explanation. Their presence would seem at first glance to indicate that elastic tension exists only in the longitudinal direction. A brief consideration will show, however, that this is not the case. After escape of the water from the enteron and collapse of the body, reduction of the circumference occurs throughout the whole piece, undoubtedly in consequence of the elasticity of the body-wall. There is, however, no physical ground for greater contraction in the transverse direction at the ends than elsewhere since there is no break in the transverse continuity of the body-

wall. The longitudinal component must cause inrolling at the cut end until either the local tension due to the formation of radial folds in consequence of the inrolling or the mutual contact of appressed portions of the inrolled wall opposes a resistance equal to the elastic tension. It is probable that in the oral half of the body, the mass of mesenteries and mesenterial filaments also oppose more or less resistance to the inrolling margins.

The flattening of the piece which often occurs (Fig. 3) is simply the result of gravity. If the collapsed piece lies on one side during several hours the weight of the body-wall is sufficient to bring about the flattening to a greater or less extent. In consequence of the flattening the openings at the ends are frequently elongated in the plane of flattening and slit-like, the inrolling occurring chiefly on the two margins of the slit.

In pieces from the extreme aboral end the inrolling at the cut margin may proceed much further (Figs. 4–6). Here the body-wall and especially the muscular layer is much thinner and must offer much less resistance to the elastic tension. Moreover, the enteron is practically empty in this part of the body. In consequence of these conditions the inrolling may proceed so far that portions of the margin are directed orally (Fig. 6).

The closure of a lateral cut by bending of the whole piece (Figs. 7–9) especially resembles a definite adaptive reaction, but can be explained as the result of elastic contraction. A cylindrical piece such as Fig. 1 does not become bent or curved so long as elastic tension on opposing sides of the body is equal. If in any way the tension on one side be reduced in effectiveness the body must bend toward that side. A transverse cut through the body-wall on one side, or the removal of a piece as in Fig. 7 interrupts the continuity of the body-wall. The longitudinal component of the elastic tension acts on the parts above and below the cut and causes contraction and inrolling of their edges. But by removal of a piece of the body-wall an open space is left and the longitudinal component of tension on the opposite side of the body causes bending of the piece so that the concave surface is on the side of the cut. The larger the piece cut out from the one side the greater will be the bending since it will continue until contact between the cut margins affords a resistance equal to the opposing tension.

Figs. 10–21 require little explanation. Here transverse continuity is interrupted by a longitudinal cut on one side. The form of the piece after inrolling is at least in large part a matter of chance, being dependent upon the relative rapidity with which the different margins roll inward.

In Fig. 11 the inrolling at the ends has been less than in Fig. 14 and the resulting form is different. In Figs. 17–21 the spiral form is due simply to the fact that one longitudinal cut margin rolled inward somewhat more rapidly than the other. An oblique spiral results from more rapid inrolling of the longitudinal margin near one end. It is clear that various conditions such as the degree of contraction of the muscles of a certain part of the body, the resistance offered by the mesenteries, the position of the piece in the aquarium, etc., may constitute conditions affecting the result.

The frequent rolling about a transverse axis of longitudinal strips cut from the body is clearly the result of the predominance of the longitudinal component of tension. It is interesting to note that this transverse rolling occurs only when the muscles are fully relaxed. If the piece be stimulated sufficiently to cause strong muscular contraction more or less complete unrolling often occurs. Pieces of this sort frequently roll about a longitudinal axis after cutting while the muscles are more or less contracted and then as the muscles relax after a longer or shorter time begin to roll transversely and continue until completely rolled up in a single or double spiral.

In cases of spiral or transverse inrolling (Figs. 17, 19, 23, 24) there is little resemblance to an adaptive reaction. As will appear, typical regeneration is impossible in these cases. Since it is scarcely to be supposed that in pieces of a certain form the reaction is adaptive in nature while in pieces of other forms it is due merely to elasticity it is preferable at least to attempt to analyze the apparently adaptive reaction. In the present case I think the analysis has demonstrated that the various methods of inrolling are all explicable on the basis of elastic contraction of the mesoglœa. The apparently adaptive character of the inrolling in cylindrical pieces where it results in more or less perfect closure of the ends is due to the particular physical conditions

present in such pieces. The inrolling of pieces after section is not then a definite reaction adapted to close the wound, except in so far as we may regard the presence of an elastic layer in the body-wall as an adaptation.

After the inrolling is completed gradual reduction in the size of the whole piece continues until the artificial openings are closed by new tissue or otherwise and the water pressure is again established in the enteron. This reduction in size can scarcely be due to the loss of tissue in the absence of food, for that is much less rapid. The piece appears to contract continuously after collapse and closure and if the closure and distention with water is prevented in any way, becomes much reduced within a few days. Frequently new wrinkles or folds appear as the contraction progresses, indicating that it is not due to actual loss of material but to some other cause.

There can be no doubt that this reduction in size of collapsed pieces is simply a continued reduction in the surface area of the tissues resulting from mechanical conditions. It is due at least in part to the elasticity of the body-wall (or especially of the mesogloea). This being effective in all directions must cause gradual reduction in size of the whole after the inrolling of the margins is completed, unless it is counterbalanced in some way, which is not usually the case. This quality of the body-wall is remarkable; pieces kept under conditions where distention with water is impossible often contract to half the size after section and collapse. If they are then permitted to close and become distended with water they may again attain in two to three days almost the original size, provided the period during which they remained contracted was not too long. The longer the period of collapse the slower and less complete is the return to the original size. These facts indicate that in the absence of the tension due to internal water-pressure the tissues gradually rearrange themselves in accordance with the altered physical conditions. There is no return to the "normal" form unless mechanical conditions once more become normal.

In his study of *Cerianthus*, Loeb ('91) has attempted to explain the collapse of tentacles and other phenomena by loss of turgor in the cells. As I shall show in a later paper, this explanation is

wholly incorrect. The question as to whether osmotic phenomena play a part in the changes above described requires, however, a moment's consideration. As regards the inrolling after section and the reduction in size of the collapsed pieces there is certainly no reason for supposing that it is due to changed osmotic conditions. It is difficult to understand how, in a form like *Cerianthus* section of the body-wall at one level should cause changes in turgor in the cells of the whole piece or of those at a distant region unless we suppose that special stimuli producing these changes arise from the region of the cut. If this be the case then the change is not primarily osmotic but reactive. Moreover, the phenomena are so obviously due to elasticity that the search for any other explanation is clearly unnecessary.

The Rôle of the Slime Secretion in the Closure of the Enteric Cavity After Section.

As has been shown, the inrolling of the margins of the piece under ordinary conditions approximates the various parts of the cut surface, and thus reduces the size of the opening. The radiating wrinkles and folds into which the inrolling portions are thrown and the frequent protrusion of parts of the mesenteries through the opening render the closure by contact imperfect. There are always slits and angles between the various parts, through which the enteric cavity is in communication with the exterior.

In spite of this fact I have often found pieces distended with water, before any closure of the ends by new tissue has occurred. A series of experiments in which the body-wall was sectioned transversely at some level and the oral portion, still bearing tentacles and disc intact, was used, will serve to illustrate this point. In every case collapse of the tentacles and body occurred immediately after section, owing to the escape of water from the enteron, but very frequently the whole oral piece including the tentacles was again distended with water in less than an hour. Examination of the aboral cut end in such cases showed that inrolling and approximation of the margins had occurred, but frequently distinct spaces between the wrinkles could be observed opening into the enteron. If the end were spread open with

needles collapse occurred at once, but was followed by renewed extension in a short time. Pieces with tentacles and disc intact show these changes much better than others, since the phenomena of distention and collapse are especially conspicuous in the tentacles. Moreover, in these pieces the presence of the mouth permits much more rapid entrance of water than is possible in pieces with oral end removed, since in these latter there is no apparatus for forcing the water into the enteron. The rapid distention of pieces under the conditions described is made possible only by the ectodermal slime secretion which under normal conditions forms the tube.

The manipulation incidental to section of the body and the stimulus of the cut itself cause a rapid secretion of this slime during the operation and for some time after. The secretion is tenacious even when first formed and clings closely to the body.

After inrolling has occurred at a cut surface only ectoderm is visible from without and where different parts of the inrolled margins are in contact the contact is usually in part ectodermal. The slime is secreted over this inrolled portion and forms a tenacious coating which closes all the crevices between the inrolled portions of the body-wall. Thus, so far as the escape o water in appreciable quantities is concerned, the cut end may be closed within a short time after section, almost as soon, in fact, as the inrolling is completed.

If the piece is left undisturbed the slime accumulates and the closure becomes more and more complete until finally the thin membrane of new tissue constitutes the definitive closure. If at any time before the definitive closure the slime be carefully removed with needles without causing violent contraction or changes in form of the piece, collapse will occur at once, showing clearly that the slime alone prevented the escape of the water.

The Growth of New Tissue from the Cut Surface.

The closure of the ends by new tissue was briefly described in the first paper of the present series, but the conditions which determine it were not discussed.

As described, the course of the process at both oral and aboral ends in typical regeneration is as follows: First the appearance of

a thin membrane of new tissue between those regions of the cut surface which are sufficiently approximated; the growth of this membrane until the whole opening is closed; the increase in size of the area of new tissue as the piece becomes distended.

During the course of my experiments it was found that certain definite conditions are necessary for this growth of new tissue.

Mention was made of the fact that the new tissue appears first in the folds and wrinkles where two cut surfaces are most closely in contact and that from these regions it extends until closure is complete. In pieces rolled spirally (Figs. 17, 19, 24) or in any such manner that the cut surfaces are not brought into contact no appreciable growth of new tissue occurs; the cut edges heal, but may remain without further change for months.

Thus, in these spirally or transversely rolled pieces typical regeneration of new tissue from the cut surface does not occur. Moreover, this is true of all cases in which there is no approximation or contact of two cut surfaces or parts of a cut surface. Never is a thin membrane of new tissue found growing out from a cut surface and without other connections. When present it always connects two cut surfaces or the two sides of a fold where different regions of the cut surface have been approximated.

This is a point of considerable importance; indicating as it does that there is nothing in the cut surface itself which initiates regeneration, the necessary condition being found rather in the relations of different cut surfaces or their parts. *Never* do we find regeneration of the body-wall occurring in the manner represented in the diagram, Fig. 25, as a continuation with free margin of the old tissue. New tissue arising from cut surfaces always appears between two cut surfaces which are in contact or closely approximated as in Fig. 2. These surfaces become united by new tissue which then increases in amount under certain conditions, thus forming a thin membrane connecting the two parts of the cut surface. In the ordinary closure of the end of a cylindrical piece the new tissue first appears, as has been noted, in the folds and wrinkles where parts of the cut surface are closely approximated (Figs. 6–8, '03*a*), but from this it spreads rapidly until the whole space is covered and the end closed (Fig. 9, '03*a*).

The process is briefly as follows: After exposure of a cut surface some slight proliferation occurs which results in healing unless another cut surface be so near that the cells arising from both are in contact; if this is the case then organic union between the two cut surfaces is rapidly established. In the closure of the ends of cylindrical pieces this process is usually completed in a few days, but in certain other cases it may proceed much more slowly. For example, in pieces which are split down one side (Fig. 10) and in which both of the longitudinal cut margins roll inward as in Fig. 11, the process of closure often requires two months or more for completion. In such cases the longitudinal cut margins usually roll inward so far that they are not in contact. At one or both of the ends, however, the closure may occur in nearly the typical manner. From the end the new tissue begins to grow along the longitudinal cut, and as it grows actually draws the cut edges together to a certain extent. The process may be compared for the sake of illustration to that of sewing up the longitudinal slit in the piece from one or both ends. If we take, for example, a case where two cut surfaces are in contact at one point and diverge at an acute angle from this point, we find that the growth of new tissue always begins at the point of contact. From this point growth and the formation of a thin membrane continue for a certain distance along the diverging cut surfaces, the extent of the membrane depending in a given species on the angle of divergence of the surfaces. This thin membrane is itself somewhat elastic and so tends to approximate the cut surfaces in greater or less degree unless opposed by other conditions. The approximation of the surfaces renders possible a further extension of the membrane between them, and so the process continues unless at some point the cut surfaces are so situated that the elasticity of the new tissues is insufficient to bring them into contact, or near enough to permit the extension of the thin membrane between them. In such a case the process of closure must cease, as often occurs. That this is actually what occurs I have convinced myself by repeated examination of specimens cut in such manner that at least some parts of the cut surfaces were not in contact while others were. The diagrammatic Figs. 26–30 will serve to illustrate the process. Fig. 26 shows

a cylindrical piece slit down one side and represented as cut across obliquely to show the separation of the cut surfaces; the cut surfaces have rolled inward and the oblique section at the lower end of the figure shows that the inrolling along the longitudinal cut is so great that the cut surfaces are not in contact. The growth of new tissue and closure begins at the oral end (it may begin at the aboral end also) where the cut surfaces are much more closely approximated and the numerous folds afford

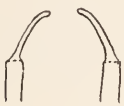

FIG. 25.

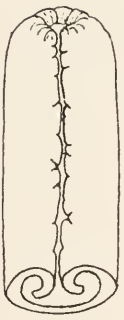

FIG. 26.

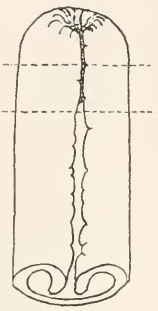

FIG. 27.

FIG. 28.

FIG. 29.

FIG. 30.

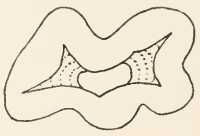

FIG. 31.

various points of contact. From this region it gradually extends along the longitudinal cut, drawing the cut surfaces together as it proceeds, as is shown by the two transverse sections, Figs. 28 and 29, Fig. 28 from a level where union has already occurred (the upper transverse line in Fig. 27) and Fig. 29 from a level just beyond where the cut surfaces have united (the level of the lower transverse line in Fig. 27). In Fig. 28 the margins have been drawn together and united, in Fig. 29 they are not yet in contact but are nearer together than at the level of the oblique section at the lower end of Fig. 27. In Fig. 30 the closure is

finally complete. In these figures only one end has been shown; usually, however, closure proceeds from both ends, and the middle portions are the last to become united.

It is evident that in a spirally or transversely rolled piece this process can never occur, for even if it begins in some fold or local approximation of the cut surfaces it cannot continue, because approximation of the cut surfaces to a degree sufficient to permit their union by new tissue is impossible as long as the piece remains spirally or transversely rolled.

Description of all possible cases of closure of pieces cannot of course be attempted. Results depend so largely on chance, that every piece affords, it might almost be said, a different solution of the problem. The examination of hundreds of pieces has, however, convinced me that the essential features of the process are those described above.

The cut surfaces appear to remain capable of giving rise to new tissue for an indefinite period. Often closure of pieces slit open longitudinally is completed only two or three months after section; yet during all this time the cut surface retains the power of producing new tissue under proper conditions, though it has no power to produce anything more than the proliferation connected with healing, provided it is not in contact with another surface. Union is as complete and perfect, though perhaps not as rapid, when it occurs two months or more after section as when it occurs within a few days.

When we compare *C. solitarius* and *C. membranaceus* we find that in the latter species the thin membranous growth of new tissue if once begun between cut edges in contact, may continue until it forms a connecting membrane between widely separated cut surfaces; in *C. solitarius*, on the other hand, the membrane is incapable of bridging over spaces so wide; the new tissue ceases to extend long before a point is reached where the cut surfaces are so widely separated. This difference is so marked that it raises the question as to whether there is a fundamental difference in the conditions and method of growth of new tissue in the two species. Figs. 6–9 of paper I. ('03*a*) and Fig. 31 of the present paper (an aboral end) illustrate the closure of openings in *C. membranaceus*. In Fig. 31 the new tissue which is

growing over the opening is in two parts which are advancing to meet near the middle. The concave free margin of both portions is noticeable. The dotted lines represent various stages in the growth of the new tissue. It is evident that it appeared first in the angles where two cut surfaces were in contact or very closely approximated. In *C. solitarius* closure by new tissue of only very much smaller pieces is possible. I believe the explanation of this difference is to be found in the different quality of the thin membrane of new tissue in the two cases. In *C. membranaceus* it is much thicker, more resistant, and less easily ruptured than in *C. solitarius*. The new tissue arises at a region where the cut surfaces are close together and may extend from this to regions where they are more widely separated. As was shown above, it exerts a certain degree of tension on the parts connected by it. As the distance between the cut surfaces increases a point may finally be reached where the tension is equal to the cohesive power of the tissue elements. Beyond this the new tissue cannot extend. In *C. solitarius* this limit is attained with a slight separation of the cut surfaces, while in *C. membranaceus* the new tissue is capable of resisting much greater tension and so of extending over wider spaces.

The membrane extending between the two cut surfaces may be compared with a fluid film bounded by lines diverging at an acute angle. The film extends a certain distance from the apex of the angle, this distance being determined with a given fluid by the size of the angle. The free margin of the film is always concave toward the opening of the angle. So long as the relation between cohesion and adhesion remains the same and the angle does not change the film can never extend beyond a certain point, since the surface-tension will cause rupture. As the angle and surface-tension decrease or as the adhesion increases the film will spread. If the arms of the angle are sufficiently pliable or capable of movement they may be drawn together by the surface-tension of the fluid, and thus permit further extension of the film.

In *Cerianthus* the thin membrane of new tissue which may be compared to the fluid film, arises at the apex of the angle, *i. e.*, where the two cut surfaces are in contact. The membrane extends along the diverging surfaces to a certain point. Its free margin is always concave (Fig. 31, also Figs. 6–8; '03*a*). The

distance to which the membrane spreads between the surfaces depends upon its composition and the degree of divergence of the surfaces, just as in the case of the fluid film. The thicker membrane with greater resistance will grow farther just as the film with less surface-tension will spread farther, other things being equal. Even the elasticity of the newly formed membrane is paralleled by the tension to which the fluid film is subjected. In both cases the margins of the space may be approximated by this tension and thus permit further spreading of the connecting film or membrane.

The illustration of the fluid film has been employed primarily as an analogy. It is not to be supposed that the thin membrane growing between two cut surfaces behaves in all respects like a fluid film extending across an angle. Yet the close parallelism between the two series of phenomena must raise the question as to whether after all the growth of new tissue from a cut surface in *Cerianthus* may not be, at least to a large extent if not entirely, determined by the laws which govern the behavior of fluids. The following facts point toward this conclusion : except so far as healing of a cut surface is concerned new tissue arises from a cut surface only when it is in contact with another, the thin membrane of new tissue which is under tension spreads between diverging cut surfaces to a certain point, beyond which no growth occurs, unless the surfaces are brought nearer together ; the point where growth ceases differs in different species, depending on the quality of the membrane ; the cut surfaces may themselves be approximated by the tension of the membrane and so further growth of the membrane made possible ; the free margin of the membrane is always concave in the direction of growth, *i. e.*, the margins of the membrane extend further than its middle region. In all of these respects the thin membrane and the fluid film behave similarly. The conclusion is at least probable that the similarity in behavior is due to the fact that similar conditions are present. I think it probable, therefore, that the appearance and growth of new tissue from the cut surfaces of the body of *Cerianthus* is governed, at least to a large extent, by the laws of capillarity. Of course the cellular structure of the tissue may complicate conditions, and the thickening and structural differentiation which

occur in the membrane after its formation bring into play other factors. These need not be considered here, however. Certainly the phenomena are far from being adaptive or teleological in any sense although the closure of the cut might appear at first glance to be an adaptation. It is difficult at present to see how they can be due to anything except simple physical conditions, though it is possible that increased knowledge may afford another explanation. Provisionally then we may regard the delicate thin membrane which appears in the angles between cut surfaces as possessing some of the properties of a fluid and as subject, at least in large degree, to the laws of capillarity.

Whether these suggestions are correct or not, the two facts above mentioned are of great importance, viz., that regeneration of new tissue from cut surfaces occurs only when two surfaces are in contact, and that the new tissue cannot extend indefinitely between diverging cut surfaces but ceases at a certain point determined by the angle of divergence of the two surfaces and the (physical) quality of the membrane, *i. e.*, is different in different species. The only possible inference from these facts is that all conditions for regeneration are not given in the living tissues themselves, nor in these plus the normal environment as a whole, but that the formation of new tissue from a cut surface is probably dependent upon certain simple physical conditions similar to those which govern the existence of a liquid film between two diverging boundaries.

Healing of the cut surface does not require these conditions; for this the necessary conditions, which are very probably also primarily due to capillarity, are established by the cut itself. The same conditions are not, however, adequate for the formation of a membrane of new tissue from the cut surface.

In this case then the conditions for new growth and closure of a wound are to be found, not in the absence of a certain part, nor in the presence of a special stimulus at the cut surface, but in simple, external, physical relations of parts. Discussion of the bearing of these facts may be postponed to another time. Attention may be called, however, to the difficulty of reconciling these facts with the neo-vitalistic theories of life and especially with that of Driesch which is based upon the phenomena of form-regula-

tion and has adopted a modification of the Aristotelian idea of an entelechy as the basis of organic form.

Summary.

1. The inrolling of the margins and the closure of openings by contact of the inrolled margins is the result of the elasticity of the body-wall. This elasticity must be greater in the inner portions than in the outer portions, in order to produce the results observed. The facts indicate that the mesoglœa plays the most important part in this elastic contraction.

2. Openings between folds of the inrolled body-wall may be stopped by the ectodermal slime secretion. This method of closure often occurs in pieces before the formation of new tissue and permits the existence of considerable water-pressure in the enteron.

3. Contact or close approximation between two cut surfaces or parts of a cut surface is a necessary condition of the growth of new tissue from these surfaces. A single exposed cut surface may heal over but no further growth occurs from it.

4. The new tissue having arisen at a point of contact between two cut surfaces is capable of extending in the form of a thin membrane for a certain distance between diverging cut surfaces; the distance to which it extends is determined in a given species by the angle of divergence of the cut surfaces, and in different species by the thickness and quality of the membrane.

5. The new tissue rising between two cut surfaces behaves in certain respects as if subject to the laws of capillarity.

Hull Zoölogical Laboratory,
University of Chicago, September, 1903.

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AN ABERRANT LIMB IN A CRAY-FISH.

E. A. ANDREWS.

A striking aberration in the form of a third, left-walking leg of a female *Cambarus Bartoni* found in class dissection in February, 1892, seems of enough interest to warrant its being put on record.

A view of the anterior face of the limb (Fig. 1) shows a markedly forceps-like structure in addition to the usual forceps at the end of the limb, so that there are four instead of the usual two terminal points.

The added structure is, however, not a true forceps with one movable finger, but a movable piece with two immobile prongs

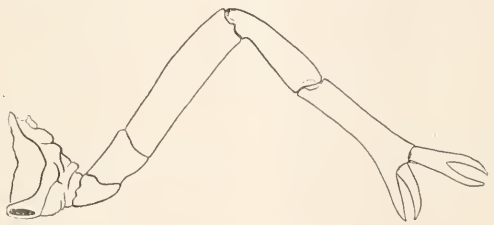

FIG. 1. Camera sketch of anterior face of left third leg of *C. Bartoni*. Genita opening indicated in black.

that otherwise resemble the index and pollex of a forceps. This is evident in the enlarged view, Fig. 2.

The real forceps in this limb is nearly normal, but on comparing it with an anterior view of the third walking leg of a normal *C. Bartoni* (Fig. 3), of about the same size, we may note some differences. Thus, in place of the straight-lined articulation of dactyl and propodite, we find the propodite presenting a hollowed, socket-like face where the dactyl articulates. Again, while the dactyl and the index are both normal in form, the dactyl is not a straight continuation of the propodite, but bends down at a noticeable angle, thus increasing the wide divergence of the double set of tips of this limb.

The propodite departs from the usual form in being wider distally where it bears, as it were, a large protuberance that is

truncated to articulate with the unusual pronged structure or second claw-like ending of the limb. There is also an abnormality in the propodite, indicated in Fig. 2, and suggesting some former injury; it is a slight indentation upon the middle of the anterior face.

The movements possible to the dactyl in the normal, alcoholic specimen (Fig. 3) are a swinging of 4.11 mm. in one plane to

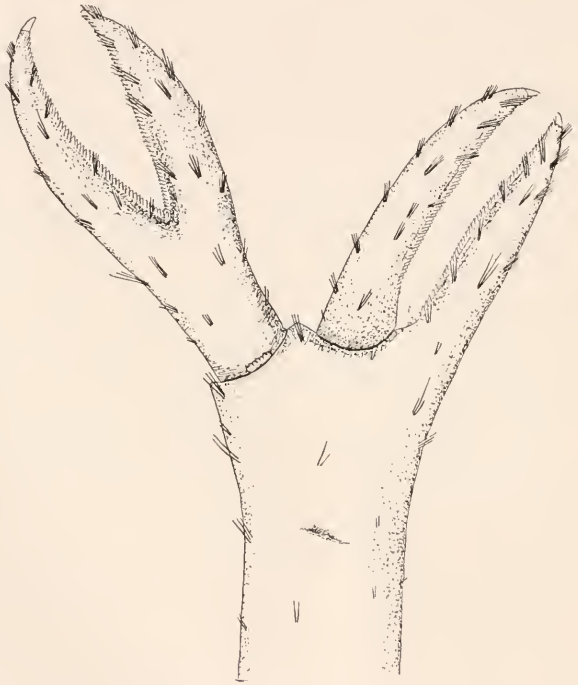

FIG. 2. Anterior view of terminal part of Fig. 1. Camera. Zeiss 2, a.

bring about direct apposition of the tips of the forceps, with no overlapping and also a very slight lateral movement.

In the aberrant limb the dactyl is so set that apposition is imperfect; the dactyl passes the tip of the index by about .5 mm. while the entire swing is the same as above, 4.11 mm. The actual gape of the forceps is restricted to that same amount, .5 mm. All movement in this forceps is strictly in one plane.

Next considering the monstrous, pronged structure we find that, starting from the position shown in Fig. 2, there is a possible

movement of 2.5 mm. in the plane of the real forceps, one half of this being towards the forceps and one half away from it. There is also mobility at right angles to the above plane, in a general antero-posterior direction. Anteriorly this is 1.5 mm. and posteriorly about .7 mm., a total swing of 2.2 mm. On moving the abnormal structure and the normal dactyl as far as possible toward one another they came just into contact. The angle of divergence between the index and the most remote part of the abnormal structure is greater than the extreme opening of a normal claw.

The form of the pronged structure is remarkable for its symmetry: the two prongs (Fig. 2) differ but slightly in shape and in size. But the one standing nearer to the claw is slightly

FIG. 3. Camera sketch of an anterior view of a terminal part of the third left leg of a normal *C. Bartoni*.

thicker and less sharply pointed and it also lacks the clear, perforated horn-like tip present upon the other prongs as upon normal claws. That this tip was lost by wear or by accident seems evident from the rough surface ending the prong and from the fact that staining liquids easily enter at this point.

Since the anterior and posterior faces of the prongs are not alike one could not put either prong into a space of the shape of the other prong; the two prongs are symmetrical about a plane between them; they are mirror images of one another, except for the above noted difference in form. This symmetry extends to such details as the distribution of clusters of bristles or hairs and to the arrangement and number of the serrations along the opposed faces of the prongs.

This latter detail deserves special description. These serrations are like those along the opposed faces of the dactyl and the index and they add greatly to the *impression that the pronged structure*

is in some sort an imitation claw. On both dactyl and index is a long series of transverse plates closely crowded together and freely projecting to give the serrated appearance noticeable under a low power. Each plate is itself serrated near its tip but these fine serrations are seen only with a higher power. As these plates stand nearer to the posterior than to the anterior face of the claw, they are more readily seen from a posterior view. Each plate, like these in Fig. 4, stands obliquely transverse and is shaped like a scalene triangle with bluntly rounded apex. It is just below this apex that the outer and distal edge bears a series of sharp teeth. This fine serration is on the edge that faces posteriorly as well as distally and the plates overlap one another so that the teeth could not be seen from an anterior view, such as Fig. 2, were it not that the plates are so transparent that the teeth can be seen through the next overlapping plate. Each plate has a central canal that passes from the epidermis through the length of the plate and ends at the surface in the blunt apex: it passes by the serrations without any connection with them. Morphologically these plates seem to be flattened setæ or hairs. In this claw there are 61 plates on the dactyl and 67 on the index; three or four are broken. As seen in Fig. 2 the series of plates is longer on the index where the proximal six or seven plates are opposed by a bare space upon the dactyl. With this exception the plates of the index and dactyl correspond, each plate having its duplicate in the opposite series.

In the pronged structure there are two series of serrations showing this same symmetry; some of each series are represented in Fig. 4.

To save space the two series are drawn as if close together while in reality the rigid prongs always held the two series far apart (Fig. 2). The plate marked T is about the thirty-fifth one from the tip of the prong that has a perfect terminal spine. The edges with teeth are those nearer to the tip and also those farthest away from the serrations of the opposite series.[1] The edge turned toward the plate of the opposite series is smooth and free. The third edge of the triangular plate is the line of attachment and is

[1] The use of these serrated plates may be to aid in cleaning the animal rather than to aid in preparing food by acting against the opposed series; they may be like the combs on the legs of certain birds.

somewhat anterior to the free tip of the plate. The terminal plates are smaller than the others and have a smaller number of teeth. This number may be as high as twelve toward the middle of the series. In all these respects the plates of the monstrous growth agree with those of the real claw. No difference was found between single plates of the dactyl and the index, nor between plates of the two prongs nor between plates of the normal and abnormal growths.

The pronged structure, however, differs from the natural claw both in number of plates and in their arrangement at the angle. While the normal claw has 61 and 67 plates the pronged struc-

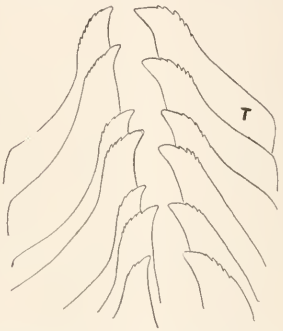

FIG. 4. Camera sketch of posterior view of serrated plates from the abnormal pronged structure. 2-D.

ture has 53 on the prong with a broken tip and 54 on the other. At the angle (Fig. 2) the plates have the arrangement shown in Fig. 5 : the terminal plates are crowded together and the two series interfere at the angle. Plate 52 of the imperfect prong steps out of rank and stands partly in between plates 53 and 54 of the series on the perfect prong, which is indicated by the letter T on the fifty-second plate. The angle thus has plates of both series carried into it till they fuse into one curved line. Moreover, these plates at the angle are not the same as the terminal plates of the normal claw, nor do they agree in number of teeth with plates at that distance from the tip of the normal claw. They are evidently special terminal plates in their own series but not directly comparable with the normal terminal plates. The prongs are shorter than the index and the dactyl and have not room for a full number of plates. Where they have a free edge

it is set with plates at the same rate as on an equal length of claw edge. If the common base of the prongs were to be split for about two fifths of its length and the prongs so lengthened they could bear about seventy plates each and the prongs would be much more like the normal claw; still it would be necessary to transform the present terminal plates at the angle into plates of the right character in the new series and to make new terminals at the new proximal ends.

But little was made out regarding the internal anatomy of this abnormal limb either from preparations cleared and stained or from sections; but it was evident that the muscles in the propo-

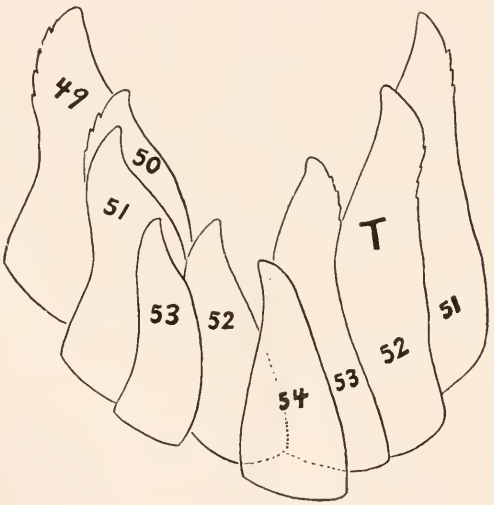

FIG. 5. Camera sketch, 2-D, of a posterior view of the plates at the angle between the two prongs.

dite were not arranged as in a normal propodite. At the distal end there were two muscle masses that seemed to connect with opposite edges of the articular end of the pronged structure. These would probably move this structure up and down in the plane of the claw. The usual muscles of abduction and adduction seemed to be developed, but attached to the dactyl in an abnormal way in connection with the above extra muscles and with abnormal widening of the distal part of the propodite.

The gist of the above description is that this abnormality is a case in which the propodite is to some extent double and bears a

normal claw as well as a pronged structure that simulates a claw even in details and was probably movable after the manner of a dactyl. This pronged structure is remarkable for its symmetry.

Comparing this with other described cases we find in the first place that it is unusual in being upon a walking leg. Of the thirty-one cases of abnormal appendages quoted by Bateson,[1] two are of antennæ, four are of non-chelate legs, and all the rest of chelæ except one, which is of a chelate walking leg.

Of the eleven additional cases given by Herrick[2] only two are of walking legs. However, this relative infrequency of described abnormalities in walking legs may be due, in part, to the greater ease with which other cases are collected or noted.

In the second place, it is unusual in being a monstrosity of the propodite. Bateson found the greater number of cases of repetition of parts, in the Crustacea, are repetitions of extra dactyls upon a normal dactylopodite (some fifty cases), and that next in frequency are the cases of extra index upon a normal index (some fifteen cases).

In the third place it seems to fall into none of the four categories established by Bateson, but rather to be like the exceptions, of which he found only two.

It has, however, resemblance to the case 815 of Bateson and more to the case shown in Fig. 195 of Herrick and still more to the case shown in Fig. 2 of Faxon,[3] which was described as follows: "This leg is provided with two chelæ. One of them has the ordinary form and structure, but is bent at a strong angle with the long axis of the leg. The second appears to have been budded off from an amputated surface of the propodite. It consists of two fingers which have the form of the normal dactylus and index, but neither is articulated with the other at the base. The two fingers together seem to be morphologically equivalent to a single segment, and represent a two-branched supernumerary dactylus."

Though the pronged structure we have described is markedly like a claw in its symmetry yet any tentative attempt to interpret

[1] Bateson, "Materials for the Study of Variation," 1894.

[2] Herrick, "The American Lobster," *Bull. U. S. F. C.*, 1895.

[3] Faxon, "On Some Crustacean Deformities," *Bull. M. C. Z.*, VIII., 1880.

it morphologically would seem to meet with more difficulty in assuming it to represent a claw than in assuming it to represent two fused dactyls or a branched dactyl. Were it a claw with fused articulation of dactyl and index we would have a limb so doubled distally as to have an extra segment and a lack of coincidence between the two series of segments. A propodite would spring from propodite instead of from a carpodite ; and if we bear in mind the partial double appearance of the propodite and regard it as a fusion we would have a carpodite and a propodite springing from a carpodite, and so on.

Bateson's thorough study led to the conclusion that almost all cases could be interpreted as repetitions of claws in which there was more or less suppression of index or of carpus. The pronged structure would then be regarded as two party fused dactyls placed face to face and we would expect to find some representative of the two indices. On the line of imagined fusion there is a slight eversion of membrane where the pronged structure articulates with the propodite, but there is no reason for regarding this as of any morphological significance.

In the case described by Faxon, as quoted above, Bateson thought he had found a representative of the required indices in a small protuberance shown in Faxon's Fig. 2 ; this however was an error for I am informed by Faxon that "the artist unfortunately represented a protuberance which does not exist."

There are thus two cases in which pronged structures have nothing with them to countenance the idea that they represent double dactyls with even traces of double indices. Moreover, it will be seen from the above Fig. 2 that the prong nearer to the dactyl is not a mirror image of that dactyl but that it represents the index and likewise the other prong is not a mirror image of the index but represents the dactyl ; this is true since all have their serrations nearer to the posterior face than to the anterior face. There is thus a departure from Bateson's rule of symmetry and we have to deal with a very unusual abnormality that is not interpretable in the same way as most of those hitherto known.

But any morphological interpretation seems somewhat premature and unsatisfactory in the lack of more knowledge of the

mode of formation of such structures. The appearance of the limb suggests a new growth following some injury in which the material for claw making was partly severed and displaced. This might happen, we can suppose, not only in the egg and in the young, but in the adult, especially at the periods of shedding when the interior of the claw is soft and the blood peculiar. That limbs may regenerate from a peripheral wound was shown by Herrick for the tips of the claws and by Morgan[1] for large parts of the limb. Possibly then such a monstrosity as this might arise in regeneration following an injury to the propodite.

An attempt to get experimental evidence resulted in failure, but this is what would be expected from the rarity of such monstrosities and from the difficulties in keeping the material long enough. In that attempt 103 mature *Cambarus affinis* were operated on in February. In each a deep cut was made in the carpodite of each chelate walking leg at a point corresponding to the pronged structure in this abnormal limb. In ten days many had healed, some could again use the dactyl and some had dropped the parts peripheral to the cut. Subsequently a piece was removed where the cut had been made in order to prevent such rapid healing. The breeding season then came on and after some months all the specimens had died without shedding and no new formations were found.

[1] Morgan, "Regeneration of the Appendages of the Hermit Crab and Crayfish," *Anat. Anz.*, XX., 1902.

THE REACTION-TIME OF GONIONEMUS MURBACHII TO ELECTRIC AND PHOTIC STIMULI.[1]

ROBERT M. YERKES.

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PROBLEMS AND METHODS.

The reaction-time method as applied to the study of the functioning of the nervous system has already given us certain important facts in human neuro-physiology, and it promises much more valuable results when its application to representatives of the various animal phyla makes a comparative survey of the time relations of neural processes possible. The value of reaction-time studies lies chiefly in the knowledge which they give us of the biological significance of the nervous system. "Certainly they are not important as giving us knowledge of the time of perception, cognition or association, except in so far as we discover the relations of these processes, and the conditions which are most favorable for them. To determine how this or that factor in the environment influences the activities of the nervous system, and in what way system may be adjusted to system or part process to whole is the task of the reaction-time investigator."[2]

For the reaction-time measurements which furnish the material of this paper chronoscopic methods were employed. All reactions to light were measured by means of a stop watch readable to tenths of a second, but for the reactions to electric stimuli, which were very much quicker, it was necessary to make use of

[1] From the Marine Biological Laboratory, Woods Holl, Mass.

[2] Yerkes, Robert Mearns: "The Instincts, Habits and Reactions of the Frog." *Harvard Psychological Studies*, Vol. I., 1903, p. 509 (*Psychological Review Monograph Supplement*, Vol. IV.).

an instrument readable to hundredths or thousandths of a second. For this purpose a Hipp chronoscope, readable to thousandths, was placed in circuit with the stimulus electrodes and the reaction-key. The electrodes were connected in such a way that the chronoscope circuit was made, and the record thereby started, the instant the stimulus circuit was completed. The motor reaction of the medusa in response to the electric shock served to break the chronoscope circuit, thus stopping the record. The experimenter was then able to read from the chronoscope dials the time which intervened between stimulus and reaction (reaction-time). Cattell's falling screen served as a regulator for the chronoscope.[1]

The reaction-key used in these measurements of the time of reaction to electric stimuli consisted of a frame for the support of an easily sliding rod, one end of which carried a cork disk and the other a platinum point by which the circuit was completed. The movement of the medusa against the disk when a stimulus was given, caused the rod to slip upward, thus breaking the chronoscope circuit.

Reaction-Time to Electric Stimuli.

Gonionemus reacts to an electric current, indirectly applied, in from one to five seconds, according to the strength of the stimulus, and the position of the electrodes. The following averages indicate the facts. Since it was not possible to get more than four or five satisfactory reactions in series with any one animal, the averages, unless otherwise marked, are for five reactions.

I. Reactions to a 4-Mesco-cell current, with electrodes on opposite sides of the bell, not in contact with the organism. M. 1.023 sec.; M.V. 0.168 sec.; R.V.[2] 16.0.

II. Same, with 2-cell current. M. 1.489 sec.; M. V. 0.199 sec.; R. V. 13.4.

III. Reactions to a 4-cell current, with electrodes 5 mm. apart in contact with the margin of the bell. M. 0.605 sec.; M. V. 0.128 sec.; R. V. 21.

Repetition of the 4-cell stimulus at intervals of a minute causes

[1] For fuller description of the chronoscopic method used see *Harvard Psychological Studies*, Vol. I., pp. 601–605.

[2] R.V. = (M.V. × 100) / M. = Relative Variability.

a rapid lengthening of the time of reaction. Thus: first reaction, .506 sec.; second, 1.003; third, 3.607.

As compared with the reactions of the small medusa *Gonionemus*, those of the jelly-fish *Cyanea arctica* are slow. Some individual reaction-times of a single individual (*Cyanea*) to a four-cell current, with electrodes in contact with opposite points on the margin follow.

Reaction.	Reaction-Time.	Deviation from Mean.
1	1.026 sec.	.504 sec.
2	1.987	.457
3	1.324	.206
4	1.636	.106
5	1.629	.099
6	1.760	.230
7	1.200	.330
8	1.328	.202
9	1.800	.270
10	1.610	.080
Mean.	1.530	Mean variation. .248

Relative Variability 16.2.

Reaction-Time to Photic Stimuli.

The reaction-time of *Gonionemus* to increase in light intensity, as I have stated in another paper,[1] varies with the strength of the stimulus, temperature, condition of the organism, etc., from one to thirty seconds. To daylight the organism usually responds in about seven seconds; to sunlight the reaction is at first much quicker, but it rapidly lengthens as the organism is exposed to the influence of the intense light. The relation of time of reaction to intensity is indicated by the following averages: Weak daylight, 9.4 sec.; daylight, 7.0 sec.; sunlight, 5.5 sec.

Moreover, the reaction-time varies with the size, sex, and pigmentation of the individual, as well as with such external conditions as temperature, density, and chemical constitution of the medium. Increase in temperature gradually shortens the time from about 8–9 sec. at 19° C., to 2–3 sec. at 32° C. Decrease in temperature lengthens the time, until reactions fail entirely at about 10–12° C.

[1] Yerkes, Robert M., with the assistance of James B. Ayer, Jr.: "A Study of the Reactions and Reaction-Time of the Medusa *Gonionema murbachii* to Photic stimuli." *Amer. Journ. Physiol.*, Vol. 9, 1903, pp. 279–307.

Between these reaction-times of the Cœlenterata and those of most vertebrates, as well as of many invertebrates, there is a striking difference in rapidity. Whereas, the jelly-fish and medusa respond to an electric stimulus in from one to four seconds, the fish or frog responds in a fraction of a second, usually not more than one fourth, and sometimes one tenth. Observe the reaction of the fiddler crab to a shadow, and note how quick it is in comparison with the reaction of *Gonionemus* to the same change in illumination. Is this difference in reaction-time due to a difference in sensitiveness (*i. e.*, is the latency period of stimulation longer); is there a difference in the rate of impulse transmission, of central nerve processes, or of muscle contraction? Such questions should be answered by means of reaction-time investigations. The rate of impulse transmission (presumably nerve transmission) is much slower in the medusa *Gonionemus* than in the vertebrates and most invertebrates thus far studied. Furthermore, it differs for different regions of the medusa; the margin and the radial

Frog.				Medusa.			
Electric Stim. Intensity.	M.	M. V.	R. V.	Light Intensity.	M.	M. V.	R. V.
1	.301 sec.	.085 sec.	28.2	Weak daylight.	9.4 sec.	3.16 sec.	33.6
2	.231 "	.034 "	14.7	Daylight.	7.0 "	2.39 "	34.1
4	.103 "	.012 "	11.6	Sunlight.	5.5 "	1.60 "	29.0

canal regions transmit impulses much more rapidly than do the inter-radial regions. The exumbrellar layer of tissue, so far as I have been able to determine, does not transmit impulses at all.[1]

The frog reacts to such an electric stimulus as was applied to *Gonionemus* in .150–.200 sec. In comparison the medusa's reaction-time is very long; but it differs in yet another respect—it is far more variable. The reaction-times and variabilities of the reactions of frogs to three intensities of electric stimulation as determined in an experimental investigation[2] are here given for comparison with the results given by *Gonionemus* to three intensities of light.

[1] Yerkes, Robert M.: "A Contribution to the Physiology of the Nervous System of the Medusa Gonionema Murbachii. Part II.—The Physiology of the Central Nervous System," *Amer. Jour. Physiol.*, Vol. 7, 1902, p. 193.

[2] *Harvard Psychological Studies*, Vol. I., 1903, pp. 616–618.

This table shows that the relative as well as the absolute variability is higher for the medusa than for the frog. In general it is true that variability increases with increase in the time of reaction. Stimuli or intensities of stimulation which give extremely short reaction-times may be expected to give low indices of variability; similarly animals which are slow in reacting exhibit high degrees of variability. The reflex reaction is absolutely and relatively the least variable among the common types of action; the instinctive reaction is much more variable, and most variable of all in time of execution as also in form, is the voluntary reaction so-called

Relation of Reaction-Time to Region Stimulated.

As might be expected the reaction-time of *Gonionemus* varies with the region stimulated. When the electrodes are placed in contact with the margin at the bases of the radial canals the reaction is noticeably quicker than when the inter-radial regions or other portions of the bell are stimulated. The average reaction-time to a four-cell current applied to the inter-radial portions of the margin is .605 second; for the radial canal regions it is .507 second. It is not necessary, however, to make measurements to thousandths or even hundredths of a second to exhibit this fact; stimulating different regions and simply watching the responses will make clear the differences in reaction-time. Again the time of reaction to light varies according as the light falls upon the subumbrellar or the exumbrellar surfaces. It is much shorter when the subumbrella is exposed to the light (3.4 seconds as compared with 17.4 seconds for the other position).[1]

It is not at all likely that the differences in reaction-time here noted for electric and photic stimuli are due to the same conditions. The quicker reaction to stimulation of the radial canal regions is doubtless due to the higher transmission rate of the differentiated nerve tracts along the radial canals. Stimulation of any other portion of the bell causes reaction less quickly simply because the tissues transmit impulses less rapidly, since they possess less highly specialized nerve tracts. In case of the quicker reaction to light when the medusa is resting with the

[1] *Amer. Jour. Physiol.*, Vol. 9, 1903, p. 301.

subumbrellar, instead of the exumbrellar, surface toward the light, a difference in sensitiveness is apparently the cause of the difference in reaction-time. Certain organs which are especially sensitive to light are found on the subumbrellar surface of the margin, and it is when they are most fully exposed to the action of light that the organism responds most promptly to the stimulus. The rate of impulse transmission is probably the same no matter which surface is stimulated by light, but the latency period of stimulation is far greater for stimulation of the exumbrella.

These experimentally demonstrable facts clearly prove that the nervous system of the medusa *Gonionemus* consists of cells which possess irritability and conductivity in high degrees. And they further show that rapidity of reaction is directly dependent upon specially differentiated paths of conduction.

Reaction-Time of Tentacles.

A study of the reaction-time of the various parts of *Gonionemus* (tentacles, manubrium, margin, bell), when normally functioning, and when isolated by operation, throws interesting light upon certain problems in the physiology of the nervous system.

Cutting off the tentacles close to the bell causes *Gonionemus* a severe shock. If only one tentacle is cut off the usual response is a contraction of the bell, which may occasionally lead to a swimming bout. The severity of the shock, or as we would commonly say, the strength of the stimulus which *Gonionemus* receives from tentacle excision, evidently varies directly with the size of the organ. Small tentacles frequently may be cut without causing any visible reaction except slight contractions of the adjacent tentacles; large tentacles cause one or more contractions, and in general the larger the organ the greater the number and force of the contractions. Excision of the primary tentacles, those at the bases of the radial canals, apparently causes the most severe shock, for when one of them is suddenly clipped off the animal frequently swims about rapidly for a considerable length of time. In every way its reaction is more vigorous than those caused by excision of smaller tentacles or of large tentacles in other positions.

It would appear from this that the radial canal tentacle is of

special significance in the life of the medusa. And in support of this belief it is worth noting that they are almost always held differently from the others. When the majority of the tentacles of a "bell up" (exumbrellar surface uppermost) individual are resting on the bottom of the vessel, the primary tentacles are usually held slightly higher in the water than the others. They are used for attachment and for food seizing sooner than the others. These facts point toward either a specialization or a modification in function which is of interest because of its bearing upon certain neuro-anatomical facts which have been presented by Miss Hyde.[1] She finds well-defined cell-fiber tracts along the radial canals. This being the case we should expect the radial canal tentacles to have a more important and direct influence upon the reactions of the organism than have the other tentacles.

When the medusa is stimulated to motion by light the tentacles contract from .1–.2 second before the bell. At times tentacle reactions occur in the absence of a general bell contraction. As determined with a stop-watch the reaction-time of the normal

I. Reaction-Time of Normal Tentacles to Daylight.

Tentacle.	M.	M. V.	R. V.
No. 1	2.2 sec.	0.22 sec.	10.0
" 2	3.4 "	0.62 "	18.2
" 3	3.6 "	0.46 "	12.6
General averages.	3.1 —	0.43 +	13.6

II. Reaction-Time of Tentacles of Excised Margin to Daylight.

Tentacle.	M.	M. V.	R. V.
No. 1	2.5 sec.	0.16 sec.	6.4
" 2	2.7 "	1.06 "	39.2
" 3	2.9 "	0.79 "	27.2
General averages.	2.4 —	0.67 "	24.3

II. Reaction-Time of Excised Tentacles to Daylight. (Average of First Three Reactions.)

Tentacle.	M.	M. V.	R. V.
No. 1	5.3 sec.	2 30 sec.	43.4
" 2	4.2 "	1.92 "	45.2
" 3	4.4 "	1.33 "	30.2
General averages.	4.5 —	1.85 "	39.6

[1] Hyde, Ida H.: "The Nervous System of Gonionema Murbachii," Biological Bulletin, Vol. IV., 1902, pp. 40–45.

tentacle to the increase in light intensity caused by suddenly uncovering a dish containing the medusa is from two to five seconds. Reaction-time averages for three conditions of the tentacle are given in table on page 90.

A fact significant in this connection is that the excised tentacle rapidly loses its power to react to photic stimuli. To the first four or five repetitions of a stimulus it usually reacts quickly, then the time of reaction, as is shown in the series herewith presented, rapidly increases until reaction fails entirely.

SERIES OF REACTIONS OF AN EXCISED TENTACLE TO DAYLIGHT.

Reaction 1	4.6
" 2	2.5
" 3	8.7
" 4	15.6
" 5	35.0
" 6	No reaction except to
" 7	mechanical stimulation.

The reaction-time of the normal tentacle, 3.1 seconds, is considerably shorter, as would be expected, than that of the bell. Its variability is low. The reactions of the tentacles of excised margins are slightly quicker, 2.4, according to the results presented, than are those of the normal animal, but they are also more variable. The quickness of these reactions may possibly be due to a temporary increase in the irritability of the margin caused by the operation. Finally, the reactions of excised tentacles are much longer, 4.5, and more variable than are those of either the normal animal or the excised margin. This may mean that the tentacle contraction in response to light is normally initiated by stimulation of the margin, or that the ability of the organ to react is lessened by its separation from the bell. At any rate there is a marked difference here indicated in the time of reaction of isolated and normally attached organs, a difference which may possibly be an indication of a function of the central nervous system or of the special organs of light stimulation which are in all probability situated in the margin of the bell.

RELATION OF QUALITY OF STIMULUS TO TIME OF REACTION.

The motor reaction of *Gonionemus* to increase in light is much slower than that to other forms of stimuli. This is due in part

to difference in strength of stimulus, but it is of interest to enquire whether the quality of the stimulus is not of importance. We may ask, for example, whether the reaction-time to the threshold stimulus of all modes of stimulation is the same. If it is not, quality of stimulus is evidently significant. Wundt[1] presents the following figures in support of his statement that the reaction-time to the threshold intensity of all modes of stimulation is the same.

Threshold Stimulus.	Mean.	Mean Variation.
Sound.	.337 sec.	0.50 sec.
Light.	.331	0.57
Touch.	.327	0.32

The results which I have gotten with frogs in working with electric and tactual stimuli cause me to question the applicability of this statement to the reactions of all organisms. It seems highly probable that the just perceptible stimulus reaction-time is by no means the same for different qualities of stimulus. Those modifications of the vital processes which make survival possible appear even in the responses to minimal stimuli. In one case the just perceptible stimulus may cause nothing more than a slight local change in circulation, excretion, muscular action, in another it may produce, just because of the particular significance of the stimulus for the life of the organism, a violent and sudden motor reaction.[2]

Absolute and Relation Variability.

As already pointed out[3] it is generally useless to compare reaction-times with respect to variability unless the reaction-time value as well as the absolute variability is considered. If, for example, to an electric stimulus *Gonionemus* reacts in 2.0 seconds, with a variability of 0.5 sec. ; and to a photic stimulus in 6.0 seconds, with a variability of 1.5 sec., it is not correct to say that the reaction-time to light is three times as variable as that to electricity. As a matter of fact the two variabilities as such are

[1] Wundt, Wm. : " Grundzüge der physiologischen Psychologie," Fünfte Auflage, Leipzig, 1903, Dritte Band, S. 428–429.

[2] *Harvard Psychological Studies*, Vol. I., 1903, p. 625.

[3] *Amer. Jour. Physiol.*, Vol. 9, 1903, p. 291.

not equal, but when we consider the reaction-times we find that the ratio of variability to reaction-time is in each case 1 : 4. Although the absolute variability is in one case three times as great as in the other, it is 25 per cent. of the average reaction-time in both instances.

Heretofore I have expressed relative variability as a ratio (M.V. : M.), or as a percentage value of the mean (M.V. = x per cent. of M., in which case x = R.V., the relative variability.)

Obviously it is always important in comparative reaction-time work to know the relative variability of reactions; in fact it is often quite impossible to make significant comparisons of results until this value is found. For this reason I have given in every table of this paper the percentage value of the mean variation in terms of the mean. This value I have called the relative variability (R. V.). It is obtainable by the formula recently used by Myers,[1] v.c. = m.v. × 100 / av. In this formula, which as inspection shows gives the ratio (in per cent.) of m.v. to av., v.c. is a value called by Myers the variation-coefficient, and av. is the mean (M.). Supposing a reaction-time of .180 second to have an absolute variability of .020 second, then by the formula (.020 × 100 / .180) the variation-coefficient (Myers), or what I prefer to call the relative variability (R. V.), is 11.1 +. If we chose this value might be written 11.1 + per cent., thus indicating that the absolute variability (M. V.) is 11.1 + per cent. of the average reaction-time (M.).

Since Pearson[2] in this statistical work has made use of a quantity which he calls the "coefficient of variability," and which is obtained by the formula

$$\text{C. V.} = \frac{\sqrt{\frac{\Sigma(x^2 \cdot f)}{n}}}{\text{M.}} \times 100,$$

it seems unwise to use the term variation-coefficient, suggested by Myers, for this new quantity in reaction-time work. As the value which we obtain by Myers' formula is in reality the per-

[1] Myers, Chas. S.: "Reports of the Cambridge Anthropological Expedition to Torres Straits." Vol. II., "Physiology and Psychology," Part II., 1903, p. 212.

[2] Pearson, Karl: "Mathematical Contributions to the Theory of Evolution, III., Regression, Heredity and Panmixia," *Phil. Trans. Roy. Soc. London*, Vol. 187, A, pp. 253–318.

centage value of the mean variation in terms of the mean, I see no reason why we should not call it the relative variability, in contrast with the absolute variability. Thus the confusion with Pearson's quantity which will inevitably result from the use of variation-coefficient can be avoided.

Myers' formula gives us precisely what we need for the direct comparison of reaction-times, with respect to their variableness, either to different stimuli or of different organisms. Strange to say most investigators of the time relations of neural processes have paid little or no attention to the variability of their results; none, so far as I know, have ever determined the relative variability throughout their work. It may be objected that those who have use for the relative variability can find it for themselves since the reaction-time and its mean variation are usually given. But the value is far too important to be left half-way determined; in fact it is even more useful in most cases than the mean variation. Every one who has had experience in dealing with reaction-time results will admit that the reaction-time to a particular stimulus has different meanings according to its variability, and that it is never possible to compare reaction-times without considering this value. It is clear then that *no reaction-time statistics should be published without determinations of the relative variabilities.*

Conventionally we compare human reaction-times to visual, tactual and auditory stimuli without noticing their variabilities or the strength of the stimulus employed. Jastrow[1] in a table of results, collected from the papers of many investigators, which is intended to indicate the differences in time of reaction for the different senses gives these averages: Visual reaction-time .185 second; tactile, .148; auditory, .139. Not even the mean variability is given in connection with the averages. Since reaction-time varies with the strength of the stimulus it is possible by varying the stimulus-intensity to get any one of the above reaction-times with any of the qualities of stimulus named. This being true, how are we to make valuable comparisons of reaction-times to different kinds of stimuli?

As before stated the threshold intensities of all modes of stimulation may be regarded as directly comparable. No matter what

[1] Jastrow, Joseph: "The Time Relations of Mental Phenomena," New York, 1890, p. 11.

the form of the stimulus, the threshold gives the longest and most variable reaction-time which can be obtained by the use of that particular quality of stimulus. Now, as the intensity of the stimulus is increased the variability decreases. Why may we not choose equality in relative or in absolute variability as a basis of comparison? If it should be found—and I am now gathering data for the settlement of the point—that the relative variability is the same for the threshold reaction-time to all qualities of stimuli, equality in relative variability would be the most satisfactory basis; if, on the other hand, absolute variability is a constant quantity at the threshold, it should be used in preference.

NOTE.—Reasons have recently appeared for returning to the original spelling of the name of the medusa. *Gonionemus* therefore is used instead of *Gonionema*.

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BIOLOGICAL BULLETIN.

THE SPECIAL PHYSICS OF SEGMENTATION AS SHOWN BY THE SYNTHESIS, FROM THE STANDPOINT OF UNIVERSALLY VALID DYNAMIC PRINCIPLES, OF ALL THE ARTIFICIAL PARTHENOGENETIC METHODS.

E. G. SPAULDING.

The genesis of this paper is a twofold one. In the first place the careful perusal of the literature which has appeared in very recent years on the matter of the obtaining of artificial parthenogenesis in various forms by a number of methods, and which includes also varying theories of the process of segmentation as interpretations of these data, this perusal readily convinces one that such a consistent and far-reaching synthetic view of the nature of segmentation as known data would seem to warrant one in trying to obtain is quite lacking. In fact no attempt seems to have been made to show that all the methods employed must result in bringing about one and the same series of physical events in the cell preceding and during segmentation, to which the process resulting from normal fertilization is no exception. That vital (?) phenomena can be reduced to a purely physical basis will doubtless be disputed as long as any details connected therewith remain unstudied or in any way ambiguous. The absence of such a complete reduction is in itself, however, no disproof of the correctness of the view as a theoretical standpoint, and success in it will at least always remain a scientific ideal.[1] A clear and detailed demonstration that the effectiveness of the various artificial parthenogenetic methods can be explained if it is held that one series of physical events always occurs in the process of segmentation would seem therefore to go a considerable way in

[1] The position that this standpoint is logically necessary for biology as a science is discussed in the author's article, "The Contrary and the Contradictory in Biology; a Study of Vitalism," in *The Monist*, July, 1903.

the attainment of that ideal. To attempt to do this states accordingly the purpose of this paper.

The second genetic element was the desire to get such a unitary view as at least a preliminary to and if possible a justification of the attempt to initiate segmentation by new methods, viz., by the application of the electrical current to the unfertilized eggs of the starfish, and although these experiments were unsuccessful, the theory, although based on the experimental work of others, is offered for what it may be worth as an endeavor to gain an end, the value of which in itself will not be denied.

1. Experimental Data.

A brief recapitulation of the results already obtained by artificial parthenogenetic methods may, as a preliminary to subsequent discussion, be pardoned.

In the starfish egg parthenogenesis may be produced by: (1) the use of HCl;[1] (2) increasing the osmotic pressure of the surrounding medium;[2] (3) by lowering the temperature;[3] (4) by mechanical agitation.[4] By the first method it is held that the "parthenogenesis of *Asterias* eggs is to be produced by means of specific (hydrogen) ions," at least this is the interpretation of the fact that 100 c.c. of sea water plus 3–5 c.c. $N/10$ HCl acting for from 3 to 20 minutes on the eggs, which are then removed, brings about the desired result.[5] In the case of the second method,[6] although the results are stated somewhat ambiguously, the maximum number of parthenogenetic eggs seems to have been secured by using 15 c.c. of $2\frac{1}{2}$ N KCl + 85 c.c. of sea water at about 23° C. for 15 minutes, then transferring. As for the third, "eggs of *Asterias* may be made to develop parthenogenetically by exposing them for a definite length of time to a temperature of 1°–7° C., in sea water, and then raising the temperature." As an interpretation of this, we find it stated that "the produc-

[1] Loeb, Fischer u. Neilson, *Archiv für die geschichtliche Physiologie*, Bd. 87, 1901.

[2] Greeley, A. W., Biological Bulletin, IV., 3, Feb., 1903, says that Neilson found this method successful.

[3] Greeley, A. W., *Am. Jour. of Physiology*, VI., 1902, p. 296.

[4] Mathews, A. P., *Am. Jour. of Physiology*, VI., II.

[5] Loeb, Fischer u. Neilson, *loc. cit.*

[6] Greeley, *loc. cit.*

tion of artificial development by lowering the temperature is brought about by an extraction of water from the protoplasm, just as if the eggs had been placed in a solution of higher osmotic pressure than that of the sea water," [1] though no explanation of the reason for this is offered. A suggestion as to this is however made by Mathews in his comments on the fourth method, that "the getting of parthenogenesis by agitation may be due to a dissolution of the nuclear membrane, since the centrosome originates close to the nucleus, or it may cause the eggs to lose water like the cells of sensitive plants. The loss of water could be caused only by lowering the osmotic pressure in the cell, and this by decreasing the number of molecules in the cell; and this in turn by synthetic processes." [2]

In other forms artificial parthenogenesis may be obtained by similar or slightly different methods; *c. g.*, in *Arbacia* by osmotic pressure, 50 c.c. $\frac{20}{8}$ *N* $MgCl_2$ or NaCl + 50 c.c. sea water,[3] and at least a segmentation by lack of oxygen, by heat, or by exposure to alcohol, chloroform, or ether;[4] in *Chætopterus* likewise by the use of KCl, KNO_3, K_2SO_4 (2½ *N* + 100 c.c. sea water), NaCl, $MgCl_2$, $CaCl_2$ and sugar,[5] in *Amphitritus* by $Ca(NO_3)^5$ (2 c.c. *N* + 99 c.c. sea water);[6] in *Nereis* by osmotic pressure, (20 c.c. 2½ *N* KCl + 80 c.c. sea water, 30 minutes),[6] in *Podarke obscura* by use of the same solution.[7] As theories and interpretations of the results obtained by these factual methods, we find in addition to those already cited the following, which are quoted in abstract:

"All that the spermatozoön needs to carry into the egg for the process of fertilization are ions, Mg, K, HO or others, to supplement the lack of the one or counteract the effects of the other class of ions in the sea water, or both. The ions and not the nucleins in the spermatozoön are essential to the process of

[1] Greeley, *loc. cit.*

[2] Mathews, *loc. cit.*

[3] Loeb, J., *Am. Jour. of Physiology*, Vol. III., Nos. III. and IX. and Vol. IV., IV.

[4] Mathews, A. P., *Am. Jour. of Physiology*, IV., VII.

[5] Loeb, J., *Am. Jour. of Physiology*, IV., IX.

[6] Fischer, M., *Am. Jour. of Physiology*, VII., III.

[7] Treadwell, BIOLOGICAL BULLETIN, III., 5.

fertilization ;[1] or the spermatozoön may carry enzymes." "Either of these two causes affects the most important qualities of life phenomena, *i. e.*, causes the proteids (1) to change their state, or (2) to take up or lose water."[2] Further details as to these two possible events are not given, however, but it is quite evident that the two may be coincident, so that the latter change may take place in any case.

To summarize systematically, a cell division can be caused in various forms by one or more of the following classes of stimuli: (*a*) mechanical; (*b*) heat (or cold); (*c*) osmotic; (*d*) chemical, or, if one will, ionic; the third for the reason that either of the electrolytes MgCl, or NaCl, or the non-electrolyte sugar, may be used for *Arbacia*;[3] therefore no specific chemical effect is to be accepted here. The fourth, a distinctly chemical effect, is evident, for HCl is effective for *Asterias* eggs and KCl is not; so also only the Ca ion for *Amphitritus*. Here then it is the kation that is considered to cause the segmentation, but that a fundamental chemical effect different to an ionic, *i. e.*, electrical charge effect is present is shown by the fact that, keeping the osmotic pressure and the number of charges on the kation the same, but changing the ions, the effect is different. This is confirmed by the comparison of the action of KCl and NaCl on muscle.[4] A specific chemical effect is therefore not done away with even if the difference in effect is reduced to a difference in the path of the charge moving around the atom. For the cause of this latter difference must in turn be a fundamental difference in the atoms themselves. The same kind of proof of an irreducible and ultimate chemical difference is found in the results of Lillie's work on the effect of Na, K, Ca and Mg salts on *Arenicola* and *Polygordius*, and of Mathews on the different stimulating effects on the nerve of NaCl, NaBr, NaI and NaFl. This fundamental chemical difference is related to the difference in solution tension, as Mathew's work this past summer has shown.

However, not alone the stimuli, the external agents initiating

[1] Loeb, J., *Am. Jour. of Physiology*, III., III.
[2] Loeb, J., *Am. Jour. of Physiology*, III., IX.
[3] Loeb, *Am. Jour. of Physiology*, IV., IV. and III., IX.
[4] Loeb, *Am. Jour. of Physiology*, III., VIII.

segmentation are to be put into the above classes, but also, with the addition of the class, surface energy, which is of special importance here, the phenomena taking place within the cell itself, preceding and during cleavage. We accordingly consider the cell to be a physico-chemical object, whatever else it may be, and subject therefore to general physical principles. The special nature of the physical processes that occur in it is to be demonstrated by showing that the effectiveness of the physical methods used for causing segmentation implies that, or at least can be explained, if by each method only one and the same series of events is made to take place. The bringing together of results in this way exemplifies what we have termed synthesis, and the internal agreement with which it is identical makes for the probable correctness of our theory.

2. General Physical Principles to be Observed in the Interpretation of These Data.[1]

If the energies both within and without the cell belong to the classes named, we must in our endeavor to get at the meaning of the data at hand be guided strictly by the most general fundamental chemical and physical principles valid for those. These principles, some of which are of course well known, may be stated as follows:

I. The "first law" of energetics, that of the conservation of energy. This is considered to have an experimental basis in the fact that, *e. g.*, a weight of one kilogram falling 424 meters raises the temperature of one kilogram of water 1° C. as indicated on an arbitrarily selected scale. This is interpreted to mean that the kinetic energy of the falling body is quantitatively equal to the heat energy gained in the rise in temperature. However this cannot be strictly proven, for the two energies are qualitatively different, and have no common factor. It would therefore be

[1] The principles as stated are to be found in no one author, but are with their criticism the result of the study of the works of Planck, Mach, Ostwald, Helm, Wald, Riecke, and others; some of these are as follows: Planck, "Prin. d. Erh. d. Energie"; Ostwald, "Vorlesungen über Naturphilosophie," "Allgemeine Chemie," and other writings; Helm, "Die Energetik nach ihrer gesch. Entw."; Rankine, *Philos. Mag.*, 1867 (4); Mach, "History of Mechanics," "Wärmelehre," "Pop. Lect.," "Analyse der Empf"; Riecke, "Lehrbuch d. Physik."

quite as logical, though not as practical to interpret the two as quantitatively different. To interpret as equal is therefore to base the law of conservation on an assumption not proven, yet not disproven.[1]

In the second place the law is based upon the impossibility of a system's continuing to do work unless energy is received from without. In this the assumption is implicitly contained that work cannot be created *ex nihilo*. Were it possible, however, for a system to receive from without the work (energy) which it itself does, a perpetual motion would be possible.

II. The second law prevents this. In every "Ausgleichung" or transformation (Umgleichung) of energy, some heat is produced, only part of which at best is again available, for the reason that it tends to "dissipate"; it cannot pass from the body of the lower to that of the higher potential (temperature) but only conversely. The entropy of the universe is accordingly said to increase. This characteristic of heat energy is a special case of a law (the second) valid for all the energies. Its meaning is that all events have a definite direction.

According to the first law then if one form of energy disappears another form or forms held to be quantitatively equal to it must appear. Energy may therefore be defined as that which in changing conserves itself. Implicit in both the first and second laws is the definition of it as that which does work, but that there are objections to this is evident from the facts stated in the second, that some energy in the form of heat with no difference of potential (T) cannot do work.

III. The factors of energy. Already present though unrecognized in the early development of mechanics, but made explicit first in thermodynamics, and later extended to all forms of energy is the view that each is made up of the product of two factors, a potential or intensity, on the one hand, and an extensity or capacity factor, on the other. In heat energy these factors are respectively temperature and entropy Q/T (specific heat), in kinetic energy V and MV, in volume energy (gases and solutions)

[1] This procedure illustrates the necessary dogmatism of all science, and the superiority of a pragmatic to a logical justification, a subject which will be treated at length in another paper.

pressure and volume, in surface energy surface tension and surface, in chemical energy chemical intensity (avidity) and mass.

IV. The law of events or action. Every event, *i. e.*, the going over of energy from one body to another or the transformation into another form is conditioned (*a*) not by the absolute quantity of energy involved, but by the potential factors which (*b*) must be opposed to each other in direction and be of different value, *i. e.*, a potential difference must exist; (*c*) this potential difference must not be compensated by a third potential, *i. e.*, must be uncompensated. Unless these three conditions are fulfilled a system remains in its state of equilibrium; if they are given between a system and its environment work is done either on it or by it, the amount of which depends upon the product of the total extensity into the potential difference. If such conditions are held to exist wholly *within* a system so that change occurs in it, this is equivalent to dividing the system into environment and smaller system, the limitation of which in every case is arbitrary though for practical purposes necessary.

V. The *direction* of the energy transfer is always from the higher to the lower potential, the one falling as much as the other rises until equilibrium is reached. By this event however a new potential difference between a second and a third energy form may be created, and with the getting of equilibrium by this a series of events is formed. Assuming the potential of a second energy to have increased or to be *continuously* increasing, the result is that in the "Ausgleichung" between this second and a third within the system, the second may be of the same intensity at the end as at the beginning of this event, while the third shows a rise in potential. This kind of event during which one potential is kept constant is called isocyclic. In comparison to the third the second potential presents here a relative fall. Conversely those events in which the extensity factor remains constant and the intensity alone changes are called adiabatic. Both kinds of changes can be brought about by manipulation of a system which is isolated with the exception of the manipulation. In natural events, however, there is always a change of potential as well as of extensity. The process of getting equilibrium is quite consistent with an absolute rise in two potentials

within a system in relation to a fall in a third potential outside of the system; at the same time the extensity factors of the first two may diminish. Under these circumstances work is done on the system. Conversely, two potentials within a system may get equilibrium and, at the same time, both decrease absolutely in relation to a third external, which rises. The system then does work. Both of its extensity factors may however increase in this process, but this does not necessarily mean a gain in energy, for energy equals potential × extensity.

Concrete instances of these possible cases, which have been selected as bearing directly on our special problem, we shall find in the physical events making up the process of segmentation and cleavage.

3. Surface Tension and Osmotic Pressure.

In the instance of the normal progress of the event of segmentation, *i. e.*, with a cleavage of both nucleus and cytoplasm, no matter whether this is the result of natural or artificial fertilization, it is an undeniable fact that, coincident with and as a culmination of all the processes taking place in the cell there is a decrease in surface tension in at least certain parts of the surface. This follows necessarily from the change in the radius of curvature of the approximately spherical form of the egg to the increased radii of certain parts of the surface of the *constricted* form. For surface tension, the potential of surface energy, varies inversely with the radius of curvature, and is greatest in the spherical form. From this it follows that in cleavage there is in any case an *average* decrease in surface tension accompanying the redistribution of this. The very fact that these changes occur may be advanced as a proof that in protoplasm we are dealing with either a solution or a fluid. Accordingly the surface energy is to be considered as due to or identical with the "attracting forces" (cohesion) of the fluid particles. This redistribution of surface tension may be correlated with and is doubtless confirmed by observed protoplasmic streamings.[1]

It would not, however, occur of itself; it must have an ultimate cause, either within or without the system (the egg), and in either

[1] Cf. Bütschli, "Protoplasm."

case is possible only if it is either the immediate *result* or *cause* of a change in the potential of that energy which is opposed to the surface tension, viz., osmotic, *i. e.*, the "repelling" forces of the substance in solution.[1] The change in surface tension is one part of the "Ausgleichung" of the potential difference existing between it and the potential opposing it, viz., osmotic pressure. The necessary coexistence of these two kinds of energy in a solution and the characteristics of each bring it about however that when one potential decreases the other does also, although there may be a relative fall of one and rise of the other, thus making an isocyclic event possible. For just as the surface tension decreases with an increase in surface and conversely, so also is a decrease in osmotic pressure accompanied normally by an increase in volume (and therefore of surface) and conversely.

It is evident then that in segmentation with its change of form and redistribution of surface tension we are always dealing immediately with the interrelations of two energy forms, surface and osmotic, and mediately with any causes which may act on them, whatever these may be. If we succeed in showing that the various physical agents used for producing artificial parthenogenesis can be effective only by in any case causing changes in either one or both of these energies, then we shall have obtained that unification of evidence which is our purpose.

4. Factors Conditioning Variations and Changes in these Energies.

The existence of an uncompensated potential difference within the egg can be accounted for in two ways. It may be either the result of changes already going on in the egg, *e. g.*, the becoming active of preferments[2] and so the formation of new chemical compounds, processes of maturation, or those leading to "natural death,"[3] but this is the same as saying that such a difference is already there and that it leads to others; or the

[1] In reply to the possible objection that colloidal solutions have no osmotic pressure there is experimental evidence that they have this and are diffusible, thus showing that there is no essential difference between these and other solutions. Cf. Höber, "Physikalische Chemie der Zelle u. Gewebe," s. 43, et seq.

[2] Cf. Hofmeister, "Chemische Organization der Zelle."

[3] J. Loeb, Biol. Bull., Nov., 1902, "Maturation, Death, etc., in Asterias."

result of action between the egg and its environment, in which case the egg may either have done work, as, *e. g.*, in a medium of less osmotic pressure, or work may have been done upon it by means of mechanical agitation, heating, abstraction of water, or fertilization by a spermatozoön. Any of these possible events must take place in complete agreement with the general conditions above outlined, and will in every case of cleavage ultimately condition a change in the relation of two kinds of energy, surface and osmotic.

That this is true is shown by consideration in detail of the characteristics of these two energies. Surface energy is due to the mutual attraction of the molecules of a fluid, which here forms one part, viz., the solvent, of protoplasm. The molecules at a certain distance from the surface are each free to adjust their mean position under the influence of the surrounding molecules, the mean position being that in which each is acted upon equally on all sides, with the result that the mutual attraction is not rendered manifest. At the surface, however, if this be free, or relatively free, as is shown by experiment (*i. e.*, if a chemical difference between medium and egg exists) the molecules are virtually acted upon only by those lying internal to them. The result is a system of forces manifesting their action throughout the fluid and at right angles to the surface, *i. e.*, radially and tending to reduce the surface to the least possible area, *i. e.*, the spherical form. The surface in contact with a chemically different medium so that mixture does not take place, acts like or is in fact a membrane or film.

The factors by which variations in the attraction of these particles for each other are determined are: (1) Their chemical nature, and consequently (2) the density of the fluid, (3) the temperature, a rise in which decreases the tension, (4) the presence of electrical charges.

Osmotic (volume) energy is identical with the mutually repelling forces of molecules in a solvent, and follows the law of gases. Its two factors are accordingly volume and pressure, and in a natural event as the former increases the latter decreases. The conditions upon which variations in this depend are accordingly (1) for equal weights of dissolved substance, and equal

volumes, chemical constitution, (2) nature of solvent, (3) electrolytic dissociation, (4) heat. Changes may be brought by chemical interaction, enzymes, heat, mechanical agitation, or amount of solvent present.

In the egg, which is a system of coëxisting energies, it is then *directly* with the attracting and repelling "forces," *indirectly* with anything conditioning them, that our problem is concerned. To be sure the molecules of both solvent and dissolved substance, the solute, must be admitted each respectively both to attract and to repel. In the former, however, the attracting forces predominate as is shown by the slight effect of temperature; the repelling may therefore be ignored. In the latter the converse is the case, for the same reason.

The form and surface of the egg at any time is accordingly determined directly by these two energies, and indirectly by any of the above enumerated conditions modifying them, for any uncompensated potential differences which may rise through the action of either internal or external causes must be equilibrated with a resultant change in surface and perhaps in volume.

The valid objection may here be offered, however, that a uniform or average decrease in surface tension does not account for the change in form accompanying segmentation. This can doubtless be met by showing that, at the same time that a decrease takes place, a rearrangement also results because of the existence in the egg of localized chemical differences, *i. e.*, the egg is organized. That these exist is shown: (1) experimentally by the different staining reactions of, *e. g.*, the nucleic and cytoplasmic cells to acid and basic dyes;[1] and (2) by observed morphological differences and changes. Granted this chemical organization, localized changes in it and so of the osmotic pressure necessarily present in such a solution can be brought about either normally by the entrance of a spermatozoön, whether its action be enzymatic or chemical, or abnormally by ions, temperature, agitation, or abstraction of water. Differences in osmotic pressure thus arising may remain localized either because of membranes within the egg, *i. e.*, of further chemical differences, or by reason of the relatively slow diffusibility of colloidal particles.

[1] Cf. the researches of Picton and Linder.

The chemical changes in the solute to which they are due may in turn be accompanied by electrolytic phenomena, and so by an attraction of unlike and repulsion of like charges, so that with all of these factors taken together reasonable grounds are furnished for the understanding of the presence of irregularities in the cleavage form. The fact of this constricted form and of the possibility of its explanation on the above basis must be constantly kept in mind in the subsequent considerations. If then the final event in the series of changes leading up to segmentation is that ending with a change of form and average decrease and rearrangement of surface tension, after which a "resting stage" of relative equilibrium exists for at least some time, then this series of events must have resulted from or be in part identical with a disturbance of equilibrium within the cell before the event of cleavage. The various theoretically possible ways in which this disturbance can be brought about must therefore be presented systematically and in detail.

5. Possible Ways of Creating a Difference Between the two Potentials Concerned, Pressure and Tension.

There is, first, the possibility that the surface tension may be greater than the osmotic pressure of the moment can withstand, *i. e.*, that a potential difference in the direction of surface tension — osmotic pressure has been formed. This of course is equalized with the establishment of equilibrium, but therewith both extensity factors, surface and volume must have decreased and the cell have lost water, while both intensities, tension and pressure, however, have presented an absolute increase. A potential difference in this direction might theoretically be caused in two ways, either (1) by increasing the surface tension or (2) by decreasing the osmotic pressure, in each case keeping the other potential constant. Conversely, if the osmotic pressure is first increased, *e. g.*, by an analytic chemical process resulting from oxidation, or if the surface tension is first decreased by, *e. g.*, electrical charges, then a potential difference in the direction of osmotic pressure — surface tension will exist. This being uncompensated, the resulting process of equilibrating is identical with the taking place of an increase in both extensity factors and

a decrease in the intensities; but in the equilibrating of potentials the decrease in the tension is less than in the pressure, for the former is already lower; it therefore presents a relative rise.

This scheme offers a systematic mode of procedure for taking up the examination of the various methods of producing segmentation and development. However, since we are concerned only with the bringing about of an average decrease in surface tension, we shall use only the second class of possibilities.

A. Factors Decreasing Surface Tension Directly.

One theoretically possible way of decreasing the tension directly is by means of either a rise in temperature or by bringing to the surface (the poles of the egg) like electrical charges.

The attracting forces, it has been seen, may be regarded as due exclusively to the solvent, which for protoplasm, we have every reason to believe, is only water; consequently any direct decrease in surface tension by changing the solvent *chemically* seems to be excluded for practical reasons. But a rise in temperature lessens the attraction potential directly at the same time that it increases the osmotic pressure; therewith is equilibrium done away with, work being done on the egg from without. The pressure being too great for the tension, the volume must increase, with absorption of water, until equilibrium is established. The extent of surface therewith increases, but the potential, surface tension, decreases absolutely, although, relative to the pressure opposing it, it rises and the egg thus in turn does mechanical work by displacing the surrounding medium. The *direction* of these events is thus determined by the difference in potential.

With this theoretically possible course of events agrees exactly the observed swelling and liquefaction of cells when heated,[1] as well as the starting of development in *Arbacia* by a rise in temperature, observed by Mathews.[2] This constitutes the first example of factual methods agreeing with our synthetic point of view, and indicates that liquefaction consists either in increasing the pressure by analytic processes, or in absorption of water, or in both.

[1] Greeley, A. W., Biological Bulletin, IV., 3, 1903; V., 1, 1903.

[2] *Amer. Jour. Physiology*, IV., VII.

Such liquefaction phenomena do not of course in every case mean, nor are they the whole of cell division, neither do they in any case account for this except by also taking into consideration the chemical organization previously referred to. But that such a liquefaction takes place during segmentation is evidenced also by (1) the greater susceptibility of the fertilized egg at that time to ether, HCl, KCl, etc., results obtained by the author in work on *Arbacia*, and an account of which will appear in a subsequent paper, and (2) by the results obtained by Lyon this summer, which indicated a rhythm in the use of oxygen and the giving off of CO_2 by the egg. The last is at the maximum during segmentation, which accordingly points to a greater splitting of molecules at that time and a consequent increase in osmotic pressure and absorption of water.

Another theoretically possible direct cause of a decreased tension would be the presence at the surface of the egg of like electrical charges. But as this concerns our own experimental work we postpone its consideration until further on.

A *fourth* possible method here would be that of mechanical agitation, by means of which the attracting forces of the membrane of the cell would be lessened; for in a physical experiment, at least, the form is charged, the average tension therefore decreased by mechanical work done from without. By analogy this may hold good of the egg, and to it corresponds the method of agitation used on starfish eggs. Its efficacy may be further explained if it is recalled that agitation may also result in a molecular splitting and consequent increase in pressure. As this completes the possible cases under this class of methods we may pass to the consideration of the other class.

B. Factors Directly Affecting the Osmotic Pressure.

We are here concerned with the establishment of a potential difference in the direction of osmotic pressure — surface tension by directly *raising* the former. Two classes of theoretically possible methods for doing this are to be considered, by bringing about (1) analytic chemical changes in the solute and (2) physical changes of pressure.

I. *Chemical Changes in Colloidal Particles by (a) Chemical Methods.*

Other conditions such as the amount of solvent being assumed as constant, the first class of changes can in theory be caused by any agent which increases the number of molecules or of particles, *i. e.*, which causes a splitting up of these within the egg. For, as is well known, in a given volume the pressure varies directly with the number of molecules. Under these circumstances, in order that subsequently there may be equilibrium between pressure and tension, both potentials must decrease, the latter however presenting a relative rise; at the same time both the volume and surface increase, water is absorbed, and the medium outside the cell is really of smaller volume and greater pressure than before.

This series of events agrees with that which we have seen must take place in segmentation, viz., an average decrease of tension. Confirmatory of the correctness of our theory and quite agreeing with it are a number of experimental methods for obtaining artificial parthenogenesis when the direct effect can be only chemical or ionic or both, but in any case not purely electronic. Thus in *Asterias* eggs HCl is effective, but not KCl, from which a specific H ion effect is inferred.[1] So also are Ca ions alone effective for *Amphitritus*.[2] (The effect of the hydroxyl ion, or of O, can have only the same direct chemical effect primarily; by these conditions segmentation is retarded or exhibited, thus indicating that for segmentation analytic processes are essential; for, *e. g.*, oxygen in the presence of ferments means a splitting up of molecules.) The accepting of a *specific chemical effect* of these ions is not invalidated by the admission of an "electronic" effect also, but is necessitated by just such data as the above, which are the results of an application of the "method of difference." Just how this chemical effect is caused, whether directly by action on the protoplasm, or indirectly by first making certain "preferments" in the egg active cannot be definitely stated, but this deficiency does not in itself alter the correctness of the above view. The effectiveness of another practical method is thus found

[1] Loeb, Fischer and Neilson, *Archiv für geschichtliche Physiologie*, Bd. 87, 1901; previously cited.

[2] Fischer, *loc. cit.*

to be explainable upon the basis of our theory and the usefulness of the attempt to synthesize therewith demonstrated.

From exactly this same standpoint also can the effect of an entering spermatozoön be explained, whether its immediate action be chemical or enzymatic, and thus the physical processes of normal fertilization and segmentation made clear, at least in part.

(*b*) *Chemical Changes by* (*b*) *Physical Means.*

Theoretically possible physical means for producing this splitting of particles or of molecules are heat, and mechanical agitation; the former, as a rise in temperature, may be supposed to have this direct effect in agreement with well-known phenomena in pure chemistry, or, as a preliminary to this, to first make certain enzymes in the egg active; or it may be considered to have a purely physical effect, for since $PV = RT$ it directly increases the pressure and decreases the tension. All of these three effects may and probably do coexist. Agreeing with these theoretical possibilities is the practical one of starting development in *Arbacia* by the action of heat (rise in T).

In the same way can also the practical method of causing parthenogenesis, *e. g.*, in *Asterias* eggs by mechanical agitation,[1] be explained, *i. e.*, in analogy to facts in chemistry. For example, in the "diazo-compounds" mechanical shock starts analytic processes. Such compounds are accordingly said to be "metastable." At the same time that the pressure is increased by such analytic processes the potential difference thus formed may be further augmented by a decrease in the tension also caused by agitation, as pointed out above.

The effectiveness of this entire class of methods, both physical and chemical, for causing analytic changes in the solute may be explained as follows: Work is first done on the cell in strict accordance with the general physical principles stated. As a result of the analytic chemical processes taking place, the pressure is at first increased, possibly also the tension decreased at the same time. If the potential difference thus created is uncompensated by a third, *i. e.*, if the pressure of the surrounding medium has been kept constant, *e. g.*, by retransferring the eggs to sea

[1] Mathews, *Am. Journal of Physiology*, VI., 2, 1901.

water in the HCl-*Asterias* method, *i. e.*, if another potential difference exists than in the cell, the latter must in turn do work; its two potentials accordingly decrease, the extensity factors increase, water is absorbed, and as a result of new localized chemical and osmotic differences in the egg plus accompanying electrical changes, the constricted form of cleavage appears.

(*Semipermeable Membranes.*)

In the above presentation the assumption is implicitly made that the surface of the cell acts as a semipermeable membrane, so that, first, only specific ions can enter, and second, others, those within the cell cannot make their exit, while water can pass either way. Only on these conditions can osmotic pressure be truly exerted.

The question accordingly arises, if this possibility is done away with if the so-called membrane is due to, or identical with, only the surface tension of a fluid in which colloidal particles are in solution. The answer to this on the basis of the evidence at hand and to accept the position generally taken is negative, but again brings us to the matter of chemical organization. Rhumbler[1] has shown experimentally that particles in the surface are not freely displaceable, nor do protoplasmic streamings to the outside take place; this can be accounted for only by assuming a foam structure and a state of solution. This means that the apparent membrane is a result of the forces of surface tension. Furthermore, with very probable reasons for the surface being chemically different to the interior as it is to that of the surrounding medium, and with the known fact of the difficult diffusibility of colloidal particles, it is quite intelligible that at the same time that a displacement to the interior is rendered difficult the specific chemical nature of the surface film should prevent the passage through it of some ions while allowing that of others, quite analogous to the artificial semipermeable membranes of Pfeffer. The same explanation would hold good for any other apparent membranes, like that of the nucleus, within the cell whose morphological differentiation can be established.[2] All these factors,

[1] Rhumbler, "Aggregatzustand u. Physikalische Besonderheiten des Zellinhaltes," *Zeitschrift für allg. Physiologie*, I., 3, '02.

[2] Cf. Höber, *loc. cit.*, p. 47, for confirmatory evidence.

viz., local chemical differences, membranes within the cell, difficult diffusibility of colloidal particles, splitting of molecules, localized processes, resulting electrical changes, make the constricted form of the egg at cleavage with its rearrangement and average decrease of tension not only intelligible, but *a priori* probable.

II. Means for Changing the Osmotic Pressure Physically.

Purely physical changes in the repelling forces of the cell are possible, and the theoretical methods to be inferred from these are confirmed by a number of practical methods for producing artificial parthenogenesis.

(*a*) *Hypertonic Solutions.*

The first of these both theoretically and also practically possible methods is the direct raising of the pressure within the cell by first surrounding it with a hypertonic solution and then subsequently transferring it to the sea water whereupon segmentation takes place. This method is used for *Arbacia*, *Asterias*, *Chætopterus*, *Nereis* and *Podarke*, and is perhaps the most important of the artificial parthenogenetic means. Its effectiveness may be explained as follows: In a hypertonic solution there is at first an uncompensated potential difference between the pressure within and without the egg, which is equalized by the passing of water through the membrane of the egg to the medium. The relative number of molecules of solute within the egg is accordingly increased. At the same time with a decrease in the egg's radius of curvature accompanying the decrease in size the surface tension has been increasing to compensate the increased internal pressure, though this compensation never quite takes place as long as water continues to be withdrawn, but theoretically is always somewhat behind. The egg thus receives energy from without. With the transferral of the egg to sea water, which is of lower pressure than it is, a potential difference in the opposite direction next exists which is uncompensated by the tension owing to the permeability of the membrane to water; accordingly an event, viz., absorption of water, takes place until the pressure both within and without is the same. In this proc-

ess, however, both the pressure and the tension of the egg *decrease*, as our theory demands, while the volume and surface increase until equilibrium is attained between the two energies within and the pressure without. But therewith new potential differences between these and other energies, *e. g.*, chemical, may have been created, by which the series is continued up through the various stages of development.

However, in this method it is not directly evident why, instead of segmentation taking place, simply the former size of the egg is not recovered when it is returned to sea water. To account for the constriction actually occurring it is again necessary to make the assumption which is nevertheless theoretically justifiable in analogy to well-known phenomena in chemistry, that in connection with localized chemical differences some of the colloidal particles are naturally in a "metastable" condition, which is done away with and chemical changes started by the withdrawal of water, and which therefore cannot subsequently be regained.[1] It may also well be in analogy to known instances elsewhere that so-called preferments become active only on the condition of a certain degree of concentration being present and that, becoming active with the concentration here present, they initiate chemical changes which finally cause the constriction of the cleavage form.

(*b*) *Action of Temperature Changes.*

The second of the class of methods we are here considering, viz., the physical, has to do with variations in temperature. Two possibilities therefore exist, a raising and a lowering of the temperature of the medium and so of the egg.

A raising of the temperature directly increases the pressure, but it may also produce, as has been seen, a molecular splitting either directly or through the mediate action of preferments, and, as before stated, decrease surface tension. Any one or all three of the effects taken together are essential with the formation of a potential difference in the required direction; the starting of development in *Arbacia* eggs by heating may be explained in this way.

[1] Cf. Ostwald, *Vorlesungen über Naturphilosphie*, s. 271 et 353, et seq.

Greeley[1] found however that artificial parthenogenesis could be produced in *Asterias* eggs by exposing the eggs to a temperature of 1°–7° C. and then allowing this to rise, keeping them in the same sea water all the time. This can be explained as follows: A lowering of the temperature directly reduces the osmotic pressure of both medium and egg, yet increases the surface tension. A potential difference within the egg in the direction of tension-pressure is thus created, the equalization of which necessitates a loss of water. This view is confirmed by the spherical form assumed and the losing of water by many species as a result of exposure to low temperatures.[2] The loss of water as a result of the increased contracting forces cannot continue indefinitely for the reason that, by virtue of the increased osmotic pressure caused by it, the egg itself would tend to absorb water. Consequently the *three processes* must be considered to occur until there is equilibrium in the entire "system" of *egg-medium;* likewise they would continue to take place, though in opposite directions, when the temperature was subsequently raised; *i. e.*, the pressure and tension would then both simultaneously decrease and the egg absorb water. Consequently this method would agree with our theoretical demand, that the event of cleavage is always identical with a decrease in surface tension.

(*c*) *Electrical Changes.*

The third possible way of bringing about changes in the egg which are in themselves physical (electrical), but perhaps due directly to chemical causes as we have indicated, is by the use of electrolytic methods. These changes may have to do with both pressure and tension at the same time and we have referred to them previously as perhaps to be necessarily considered as present in any case in order to account for the constricted form of cleavage. For it can be shown that every method that we have analyzed may, at the same time that it results in the changes in chemical composition, pressure and tension, have also accompanying these a change in the electrical condition of the egg.

[1] *Am. Journal of Physiology*, VI., 1902, p. 296.

[2] Greeley, *Am. Journal of Physiology*, 1901, VI., p. 122; BIOLOGICAL BULLETIN, III., p. 165 and V., p. 42.

Lillie[1] in two recent papers has emphasized the importance and essentiality of these electrical phenomena for segmentation, but, inasmuch as he ignores to a certain extent the consideration of the factors we have emphasized, and because we believe it can be shown that his own view is incomplete without this, it may be allowed us to quote quite extensively and in abstract.

Lillie in his first paper finds that "the tendency of colloidal particles to collect at the electrodes indicates that they carry a surface charge, either positive or negative." Precipitation can be caused by ions bearing charges of opposite sign to those of the particles, liquefaction by those of like sign. "The researches of Picton and Linder, and Hardy indicate that the nucleo-proteids especially the chromatin of dividing cells and of spermatozoa are pronouncedly acid and therefore electronegative; cytoplasmic proteids are conversely basic and positive. Accordingly a difference of electrical potential exists between cytoplasm and chromatin in the cell, which difference is greatest at the time of mitosis, when the chromatin is most strongly acid. This potential difference may constitute the primary and determining condition of mitosis."

In confirmation of the correctness of this view he finds that "in all cases the appearance of the cytoplasmic radiations and the formation of the mitotic figure are accompanied by a passage of the nuclear chromatin into a phase rich in nucleic acid. Evidently the two parallel series of changes are intimately connected. Furthermore, the marked resemblance between the rays of the mitotic figure and the electric and magnetic lines of force are additional indications that the process is essentially electrical in nature. The position of the chromosomes during mitosis indicates a mutually repellent action similar to that of similarly charged bodies. The same action takes place in the chromatin filament whereby it assumes a coiled or spiral form."

In his second paper his purpose is to show the necessity of these electrical conditions for segmentation. Evidence for this is that "cytoplasmic cleavage in fertilized *Asterias* and *Arbacia* eggs is prevented in solutions of non-electrolytes, although the

[1] R. S. Lillie, *American Journal of Physiology*, VIII., IV., Jan. 1, 1903, and Biological Bulletin, IV., 4, March, 1903.

nuclear division continues. In these solutions the electrolytes present in the egg must diffuse outward. Cleavage therefore depends on the presence of these in the cytoplasm. Likewise a strong tendency to fusion shows itself in blastomeres transferred to these solutions during early cleavage stages; therefore further cleavage also depends on the presence of electrolytes in the cytoplasm. This action is due to the ions into which they *dissociate*."

If all this is admitted it seems to us quite necessary to admit or infer therefrom that the presence of electrolytes in the eggs means also the presence of osmotic pressure; one cannot exist without the other; consequently their dissociation is identical with an increase in pressure. This would be in perfect agreement with our own theory, but is quite ignored by Lillie. He does, however, recognize with us the necessity of accepting a decrease and rearrangement of surface tension at cleavage, which he accounts for by a difference of electrical potential between the egg and medium. For "Lippman and Helmholtz have shown that the surface tension is greatest when the electrical potential at the boundary is zero, and decreases as the latter increases, for like electrical charges at the surface oppose the tension and diminish it. From this is evident the importance of electrolytes in segmentation; for the production of a potential difference between the egg and the medium separated by a semipermeable membrane is accompanied only by a migration of ions. Therefore, there are ions within the egg originally, and a potential difference implies that ions of like sign are respectively at the surface and in the interior. This state of affairs is found at cleavage. Observation of the direction of the fibrils in the egg during mitosis agrees with this view, *i. e.*, they correspond with the lines of force.' How is this difference of potential between the surface and the interior established? The answer is that "during segmentation especially is the chromatin markedly acid, the cytoplasm basic." This means in our opinion that there is chemical organization. "Agreeing with the results of Nernst and Olsen, the chromatin represents a charged body by the action of whose negative charges the anions are repelled toward the periphery, or the poles, and the kations are attracted to the nucleus." A curren

passes therefore in the direction of the gradient from periphery to center. Such inductive phenomena resulting from the increased acidity of the nucleus during mitosis must in turn be due, we find, to the preceding chemical changes resulting perhaps from the action of ferments. Accordingly, *it results* that "the center of the astral radiations is the region of highest positive potential, the surface of negative, and hence the decrease in the surface tension by the like charges present."

But a uniform decrease in tension is no change in form; to bring this about the tension must be unequal. To explain this, Lillie says, "There are indications, *e. g.*, the elongation of the spindle axis, that the primary lowering of surface tension by the above agencies is at the two sides of the egg opposite the astral centers. From the position of the astral centers during metaphase and telophase it is to be expected that the surface negative charges are densest near regions adjoining the long axis of the egg and that there surface tension is lowered to the greatest degree. The effect in these regions will increase as the daughter groups of chromosomes approach the poles, since the inductive action increases as the distance decreases. The surface tension at the regions adjoining the astral centers must therefore decrease as the daughter groups approach the surface, *i. e.*, the difference between the surface tension at the poles and at the equatorial region progressively increases. Eventually the egg is surrounded by an equatorial surface zone possessing higher tension and acting like a constricting band; a cleavage furrow follows."

"From the fact that not all of these events takes place when the egg is placed in solutions of non-electrolytes, it is clear that cleavage depends on the presence of ions, and that in fertilization the spermatozoön carries either these necessary electrolytes into the egg or ferments which initiate their formation and action."

That the acceptance of the events either identical with or similar to those so ingeniously outlined by Lillie is necessary in order to complete the theory of segmentation advanced in our own paper, may be admitted. But that the two are not contradictory to but rather must supplement each other seems quite as necessary to admit. The constricted form of segmentation must

be accounted for, but if this is done by electrolytic theories, then the existence of osmotic pressure cannot be denied. Lillie, however, neglects its consideration and in this respect his view is incomplete. If the necessity for segmentation of the presence of electrolytes in the egg is *proven* by such data as Lillie has advanced then osmotic phenomena must be also present, and that they play an important part cannot be denied in the face also of the evidence from artificial parthenogenetic methods. Rather, *conversely*, starting with the known fact of the effectiveness of, *e. g.*, the osmotic pressure methods, the possibility of these leading to the electrical changes should be shown. The objection may be made that, although present, osmotic pressure may nevertheless be left out of consideration for the reason that it is of low intensity. This objection does not hold good, we think; for, given a semipermeable membrane and the tendency of surface tension to reach its maximum, the latter will do this until balanced by at least as high a pressure as in the normal surrounding medium, sea water, of the marine forms we are considering.

It therefore seems possible and even necessary to unite the two views; to take the position that the electrical phenomena described by Lillie cannot be done away with but, being essential, they can nevertheless, as we have experimental evidence to show, be brought about in a number of different ways, such as amount of water present, action of heat, agitation, etc.; and that they coöperate with the factors we have emphasized in making up the physical and chemical events of cleavage processes. This possible perfect agreement of Lillie's theory with our own, into which all the factual methods have been shown to fit, adds, it seems to us, one more confirmatory element to the synthesis which it has been our purpose to attain.

The electrolytic methods which have been referred to as the *third* class which bring about physical changes may therefore be explained from the standpoint of this more complete view. As our factual result we have artificial parthenogenesis produced in *Asterias* eggs by the action of $N/10$ HCl solution (2–5 c.c. + 100 sea water) for about one hour, with a subsequent transferral to sea water. Dissociation takes place in the dilute solution used.

The negative results obtained with KCl indicate that it is the H and not the Cl ion that is effective. Two views of the way in which this ion acts are conceivable. First, an interpretation we have referred to previously, it may be that only the H ions penetrate the membrane, either because of *its* specific chemical nature or because of *their* greater diffusion velocity; or, second, that, with no penetration occurring, the cytoplasmic membrane simply attracts the H ions to the surface.

Both views however present difficulties if the H ions are considered to have a purely inductive (physical) action. The first view in its very genesis, because, *firstly*, unless the surface is negative all the time it is hard to understand why the positive ions should even be attracted to it, much less penetrate it; if, on the contrary, it is at first positive they would not reach it, being repelled, unless their velocity of diffusion overcame this repulsion; but *secondly*, because if penetration does occur it is difficult to understand how at least many of the ions can reach the nucleus from which point possibly to, in turn, induce negative charges at the surface; for cytoplasm and H ions are like-charged; these would, however, tend to cause a repulsion of cytoplasmic particles and therefore an increase in pressure. The second view meets with objections because, with *no penetration occurring*, it is difficult to understand also here why, firstly, if the surface is not yet negative before mitosis, either H or any other kations should be attracted, or, secondly, if the surface is negative at all times and the cytoplasm positive, so that the surrounding H ions may induce further negative surface charges, why the H and not other kations as well should be effective. That they are might seem to be explained by the comparative diffusion velocity of H, K and Cl ions (H is 313, Cl 65.9, K 63.8)[1] from which it might be assumed that the K ions are not effective because with them the Cl ions while in the case of HCl the H ions have the greater comparative velocity. But this explanation would not hold good for explaining positive results with $Ca(NO_3)$ on *Amphitritus*,[2] for the diffusion velocity of $Ca_{\frac{1}{2}}$ is 62, that of NO_3 65.[3]

[1] Höber, *loc. cit.*, p. 72.

[2] Fischer, *Am. Jour. of Physiology*, VII., III.

[3] Höber, *loc. cit.*, p. 191.

Between the difficulties of the two views it seems necessary to conclude that *certain* ions penetrate the membrane because of *their* and *its* specific chemical nature and of their greater comparative velocity and notwithstanding the possible repulsion, and that there follows a specific chemical effect on the chemically organized cell contents accompanied by those electrical and osmotic phenomena above considered. That there is a chemical effect is indicated also by the different results obtained in determining the rhythm of immunity of fertilized *Arbacia* eggs to ether, HCl, KCl, etc., an account of which will appear in a later paper. Furthermore, from this standpoint there should be in theory no effect resulting from the use of either the anodal or kathodal end of the current on the eggs of, *e. g.*, *Asterias*, and the negative results of such experiments carried on by the author this summer are confirmatory of this view.

Conclusions and Summary.

Under the experimentally justified assumption that the organism is a peculiar complex of energies, so that general physical principles are therefore valid for it, it is found that the effectiveness of both normal and artificial fertilization methods can be explained from one standpoint, viz., firstly, that the necessary condition for the event of cleavage, is the creation, previous to it, of an uncompensated potential difference between osmotic pressure and surface tension by increasing in a chemically organized egg either absolutely or relatively the pressure or by decreasing the surface tension; secondly, that the event of cleaving is itself identical with the equilibrating and compensating of this difference, which necessiates an average decrease in both the potentials, osmotic pressure, and surface tension; and thirdly, that there is an accompanying unequal distribution of electrical charges at the surface and at the center in such a way that constriction results therefrom.

This constitutes the synthesis which we purposed, and is offered only as an attempt, that, although in itself justifiable, presents much that is incomplete and tentative.

College of the City of New York,
Department of Philosophy, December 22, 1903.

EXPERIMENTS ON THE DEVELOPMENT OF EGG FRAGMENTS IN CEREBRATULUS.[1]

N. YATSU.

The question, "To what extent can the principle of germinal localization be applied to the unsegmented egg, and how far, on the other hand, may the specification of the embryonic regions be considered a progressive process that falls under the category of epigenetic phenomena?" (Wilson, 1903[2]) led me in the summer of 1903 to carry out a series of removal-experiments on the eggs of *Cerebratulus lacteus*, during the later part of my stay at the Tufts College Marine Laboratory at Harpswell, Me. I wished to determine whether the cytoplasmic localization is progressively established, and especially to ascertain the conditions existing in the egg just prior to the first cleavage. To this end I have examined the development of egg fragments, obtained by cutting, at four successive periods between discharge of the egg and the first cleavage, namely, (1) before the dissolution of the germinal vesicle, (2) at the metaphase of the first polar mitosis, (3) at the period of conjugation of the egg- and sperm-nuclei and (4) after the constriction of the first cleavage appeared. The result shows that the percentage of abnormal larvæ steadily increases as the egg approaches the two-cell stage. When I appreciated the importance of comparing with these results the development of isolated blastomeres of the two-cell stage, the breeding season was nearly over, and I was only able to make a few experiments, which are not numerous enough to give a satisfactory basis of comparison. I hope, however, to carry on more complete experiments at the first opportunity.

A word about cutting the eggs. A drop of water containing several eggs was spread on a slide, and some of the water was

[1] For the preparation of the present paper my best thanks are due to Professor E. B. Wilson for his kindly suggestions and criticisms. I am also indebted to Professor J. S. Kingsley for kindness shown me at the Harpswell Laboratory.

[2] E. B. Wilson, "Experiments on Cleavage and Localization in the Nemertine Egg," *Arch. f. Entwm.*, Bd. 16, Heft 3, p. 440.

removed by a pipette. The egg, thus flattened a little, was cut with a fine scalpel. It sometimes proved better, in order to spread the water evenly, to use a little albumen fixative, rubbed on the slide, and thoroughly washed off with a brush. In doing so, the quantity of glycerine left on the slide was infinitesimal, so that it did not affect at all the development of the eggs. My experiments consisted in cutting off a portion of cytoplasm from unsegmented eggs at the four periods already mentioned, in the first two cases fertilizing the fragments, and rearing the resulting embryos up to the pilidium. For the sake of uniformity I have confined my work to the nucleated fragments. Cleavage was studied up to the eight-cell stage. Among the pilidia thus obtained, I found many abnormal ones, and they were compared with the normal larvæ. Every pilidium was drawn with a camera between 48 and 58 hours after fertilization. During drawing they were kept still by sucking out some of the water from under the cover-glass, which was supported by a piece of thread. Thus they could barely move without distortion of their shape. It should here be noted that defective larvæ remain always defective ; moreover, the defective parts become more and more prominent as the larvæ grow. No size regulation takes place among the pilidia ; the smaller the original egg pieces, the smaller the pilidia.

Series A.

Development of Fragments obtained before the Disappearance of the Germinal Vesicle.

A portion of cytoplasm was cut off from the egg immediately after its release and while the germinal vesicle was intact, and the nucleated piece was fertilized. Care was taken not to injure the germinal vesicle, and most of the operations were done upon eggs in which it was eccentrically situated, so as to cut off as much cytoplasm as possible. Since the germinal vesicle, as a rule, lies nearer to the animal pole, it may be inferred that most of my sections were performed in the vegetative hemisphere, although I did not record the exact plane in this series.

Thirty-five egg-fragments were able to develop up to the pilidium stage, the rest having either died or been rejected on account of polyspermy. The result may be tabulated as follows :

Perfect pilidia 30	smaller 6, larger 24	85.7 per cent.
Defective pilidia 5		14.3 per cent.

It is striking that comparatively few larvæ turned out to be defective. All of these are shown in Fig. 1, *C–G*,[1] a glance at which shows that they are certainly abnormal, yet their defect is not so great as those obtained at later periods (Series B and C).

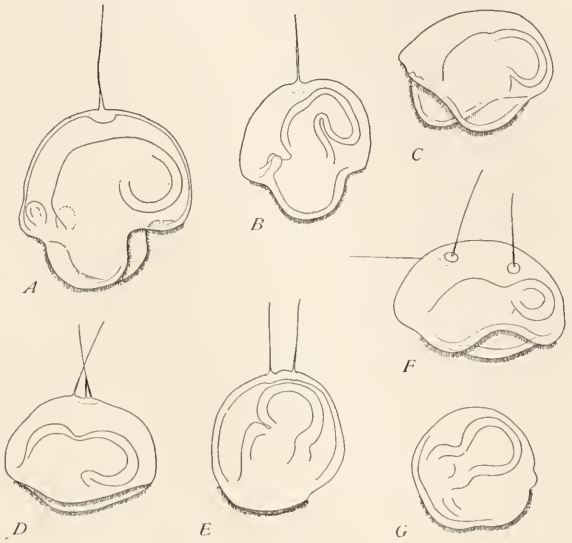


Fig. 1.

1 *C* represents a larva which would be perfect, if there were an apical organ. 1 *D* is another defective one with three apical organs, two tufts having been about to fuse ; in other respects it is perfect. A pilidium represented in 1 *E* is much more abnormal than the preceding two. It has two apical organs and a pair of ciliated lappets of smaller size. The shape of the gut also is not normal. Another pilidium (1 *F*) resembles the one with three apical organs just described (1 *D*), but differs from it in having three apical organs widely separated from one another — two on the left side, one on the right. In this larva the left ciliated lobe is indented. Lastly we have a very defective pilidium (1 *G*), which, like 1 *C*, has no apical organ. While the ciliated lobes

[1] All the figures throughout the paper have been drawn by the camera ; 84 diameters magnification.

are comparatively normal, the gut is quite abnormal, the stomach being larger than the œsophagus, and the position of the mouth deviating from the normal. Nevertheless each part is complete, except the apical organ. It should not be overlooked that the apical organ is abnormal in every one of five defective pilidia, while both the gut and ciliated lobes are fairly unaffected by the operation. From this it is probable that the basis of the apical organ is vaguely foreshadowed as early as the stage at which the germinal vesicle is still intact.

It is highly important to note that the formation of a pilidium does not depend on the size of the piece, since one fourth of the perfect larvæ are decidedly smaller than the rest. The larva, 1*A*, is one of the larger group, and 1*B* one of the smaller. Both are perfect in every respect, but one is a little larger than one half of the other. From the fact that, in spite of cutting the eggs at random, a large percentage of perfect larvæ were produced, that the perfect ones vary greatly in size, and that the defects are not so considerable as at a later period, the most natural interpretation one can draw would be that, antecedent to the dissolution of the germinal vesicle, the egg cytoplasm still shows little or no definite specification of the germ regions.

Series B.

Development of Fragments obtained at the Metaphase of the first Polar Mitosis.

The eggs were cut when the mitotic figure of the first maturation division was completed; this can readily be seen as a clear space at the animal pole. The nucleated fragment was fertilized. Sixty-five eggs thus operated were able to develop into larvæ. Owing to a change of consistency of either the gelatinous egg envelope or the egg itself, I found it more difficult to operate at this stage than at any other. The result was:

Perfect pilidia 34	52.3 per cent.
Defective pilidia 31	47.7 per cent.

Notwithstanding the difficulty of the operation, it was comparatively easy to cut off a considerable part of cytoplasm from the egg of this stage on account of the peripheral position of the

mitotic figure. Consequently in some cases the fragments were quite small. In this series all the defective larvæ were more abnormal than in the preceding. The abnormalities may be classified as follows:

Defect in apical organ.	Larvæ with	supernumerary apical organ	4	14
	" "	no apical organ	10	
Defect in ciliated lobes.	" "	ciliated lobes indented or not well developed	16	27
	" "	only one ciliated lobe	11	
Defect in gut.	" "	no gut	6	21
	" "	no œsophagus	5	
	" "	no stomach	1	
	" "	stomach cut off from œsophagus	2	
	" "	gut not well developed	7	

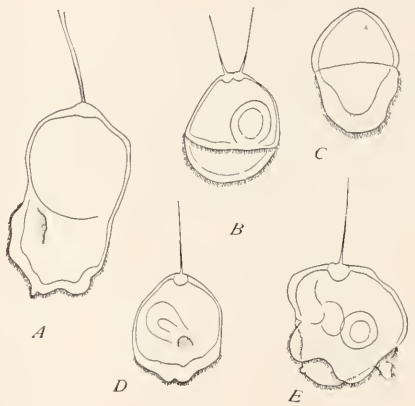


FIG. 2.

The disturbance of the apical organ (2*B* and 2*C*) is of special interest, indicating that its basis covers quite a large area over the animal pole, since in this series it was impossible to cut off cytoplasm from the top of the egg. While in Series A the defective part was chiefly restricted to the apical organ, we find that in the stage under consideration the abnormalities extend to the ciliated lobes and gut also. As will be seen from the above table, a large number of the defective pilidia have abnormal ciliated lobes; in some, one of the lobes is very short (2*B*), while in others the lobes are indented (2*E*), reminding us of *Pilidium branchiatum*. Quite commonly one lobe is entirely wanting (2*A*, 2*C* and 2*D*). The defect in the gut is remarkable. In a few larvæ

there is no gut at all, these being not far from the "Dauerblastulæ" (2*C*); in other cases the gut is represented by a shallow depression (2*A*). In some defective larvæ either the stomach or the œsophagus may be wanting (2*B*, 2*D*). In a few cases the stomach is cut off from the œsophagus (2*E*).

It is noteworthy that there occur a few defective larvæ which are as large as the perfect ones (2*A*). And among the perfect pilidia only three were smaller than the others.

From the increased number of defective larvæ in this series, it may be inferred that, since the fading of the germinal vesicle, the regional specification has advanced. But the occurrence of perfect pilidia equal in number to the defective ones cannot be overlooked. It is possible that the production of the perfect larvæ is because the injury was too small to have materially affected the organ-bases, or because the plane of section was such as not to disturb their normal proportions. The number obtained is, however, too great to lend much probability to either of these suggestions. A more likely interpretation is that the egg still possesses a considerable power of regulation.

Series C.

Development of Fragments obtained at the Period of Conjugation of the Egg- and Sperm-nuclei.

The eggs were fertilized and a portion of cytoplasm was cut off a little after the second polar body was extruded, *i. e.*, at about the stage at which the egg and sperm nuclei came to fuse. In this series the polar bodies gave a very good landmark for orientation of the egg. The accompanying cut shows the direction of section plane (Fig. 3). The eggs which were able to develop up to pilidium were not as numerous as in Series B, but they gave a fairly conclusive result, as shown in the table on p. 102.

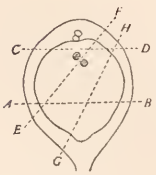


Fig. 3.

Most of the pilidia of this series are defective, as tabulated. Not only that, the defective parts correspond in a general way to the region from which the cytoplasm was cut.[1] It is very

[1] The apparent contradictory result as in the case of Nos. 17 and 23 is probably due to the volume of the cytoplasm cut off.

No. of Eggs.	Section Plane.	Condition.	Remarks.
1	AB	D(efective)	Ciliated lobes short.
2	"	D	No apical organ; ciliated lobes asymmetrical.
3	"	D	One of ciliated lobes wanting. Gut not well developed.
4	"	D	Apical organ on the right side.
5	"	P(erfect)	
6	CD	P	
7	"	P	
8	"	P	
9	"	P	
10[1]	"	D	Two apical organs. Gut double.
11	EF	D	Ciliated lobes defective.
12	"	D	One of ciliated lobes wanting.
13	"	D	One of ciliated lobes defective.
14	"	D	Gut defective.
14′	"	D	No apical organ; one ciliated lobe wanting; gut comparatively large.
15	"	D	One of ciliated lobes wanting.
15′	"	D	One of ciliated lobes wanting.
16	GH	P	
17	"	D	One of ciliated lobes wanting.
18	"	D	Neither of ciliated lobes developed, but the margin ciliated.
19	"	D	One of ciliated lobes wanting.
20	"	D	Ciliated lobes defective.
21	"	D	Ciliated lobes defective.
22	"	D	One of ciliated lobes wanting.
23[2]	"	D	No apical organ; only one lobe present; gut imperfect.
23′	"	D	No apical organ; only one lobe present; gut imperfect.

Summary:

Perfect pilidia 6.....24 %

Defective pilidia 20.....76 %

important to observe that most of the perfect larvæ were produced from the egg cut along *CD*. The basis of the apical organ must, therefore, have existed below the line *CD*. This fact, and the frequent occurrence of a defective apical organ in series *B* taken together, it may safely be concluded that the basis of this organ takes the form of a broad ring a little above the equator. But how it finally takes an apical position I am at a loss to imagine. Although Professor Wilson's experiments[3] were not directed to this problem, his results seem to tally well with

[1] In this egg the nuclei conjugated in the vegetative hemisphere; the section plane passed nearly through the equator.

[2] In this egg the sperm-nucleus must have been present in the piece which I thought enucleated.

[3] Wilson, *l. c.*, pp. 432, 433 (Fig. 10), 436.

my conclusion. He obtained through the section near the animal pole along *op* (his Fig. 3, *B*, on p. 240) the animal larva without any apical organ, the vegetative one in this case having been provided with the multiple apical organs (his Fig. 10, *E*, on p. 433). On the other hand, in another case, he found that both the animal and vegetative larvæ had the normal apical organ

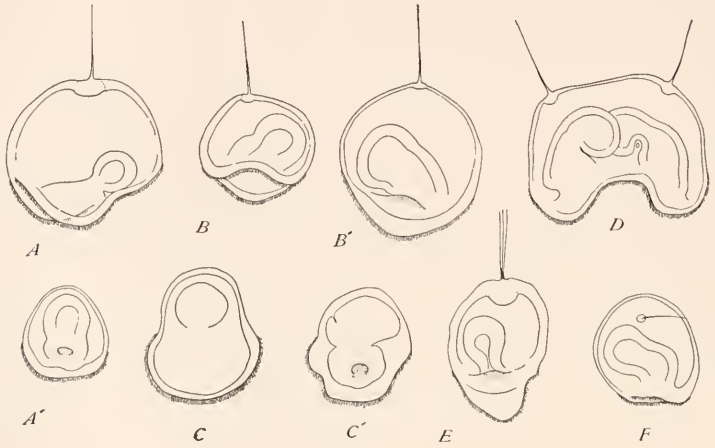


FIG. 4.

(his Fig. 10, *A* and *B*) when he cut along the plane near the equator (*kl* of his Fig. 3, *B*). In this case it may be inferred that the section plane bisected the basis of the apical organ. He gives still another instance of the animal larva with the apical organ (his Fig. 10, *C*) obtained when he cut below the equator along *mm* (his Fig. 3, *B*).

The pilidium No. 10 (4*D*) is a very important one. It was produced from an egg from which about one third of the animal hemisphere was cut off. This was about as large as a perfect larva if seen from the side, but it was extremely compressed laterally. It has not only two apical organs but two guts. I suspect that it might have been disturbed by some unknown cause at the two-cell stage because, as a rule, half-larvæ obtained by isolation of blastomeres are larger than one half of the normal larvæ. In another pilidium (No. 4) I found the apical organ shifted from the normal position (4*F*). In spite of this, it swam with the apical pole directed forward like a normal pilidium.

In three cases I got two larvæ from one fertilized egg. There are three possibilities to explain this: first, when the operation is done before the fusion of the egg- and sperm-nuclei; second, after the first cleavage mitosis came to the telophase; third, when the egg is doubly fertilized and the segmentation nucleus is cut apart from the sperm-nucleus. Since my operations were done not so late as the second case, the result may be due either to the first or the third cause. Whatever the cause may be, the comparison of the resulting larvæ is very interesting. $4A$ and $4A'$ (Nos. 14 and 14′) show a most instructive pair of pilidia. The larger of the two is almost normal, except that the gut is very defective, while the smaller one has a comparatively large gut. Professor Wilson found a similar pair of larvæ by cutting the blastulas (cf. his Fig. 11, *A* and *B*). Another pair ($4B$ and $4B'$, Nos. 15 and 15′) are also important; both of them are normal, but one ciliated lobe is wanting in each. In still another pair, $4C$ and $4C'$ (Nos. 23 and 23′), both are devoid of the apical organ and have a very defective gut. Either of them is barely more than the lappet.

Now let us see how the basis of the ciliated lobes and gut are disposed in the egg of this stage. Most of the defective larvæ have abnormalities in the ciliated lobes in some way or other. We can distinguish two kinds of defect; in one only one ciliated lobe has been developed, the other being entirely suppressed, so that the mouth can be seen from the side ($4A'$, $4B'$, $4C'$ and $4E$). The other kind of defect is shown in $4F$. In this case both the ciliated lobes are present, but they are very short and almost straight. This difference may be ascribed to the fact that the basis of the ciliated lobes is more or less bilaterally situated in the vegetative hemisphere near the equator. As for the basis of the gut I know very little, but it is certain that it lies near the vegative pole (cf. $4A$ and $4A'$).

Series D.

Development of Fragments obtained before the Completion of the first Cleavage.

The eggs were cut between the period of the appearance of the first cleavage furrow and the completion of the division. This

experiment was begun at the end of my stay at Harpswell, and I can give here only four cases.

(1) The egg was cut along *AB* (5*A*) and a perfect pilidium was produced which is represented in 5*C*. It is important to observe that, in spite of cutting off the cytoplasm from the animal pole, the apical organ has developed undisturbed.

(2 and 3) The eggs were cut along *CD* (5*A*). From each blastomere arose a pilidium with one ciliated lobe and no apical organ. The cases are too few to draw any conclusion, but the importance of the cytoplasmic bridge connecting the blastomeres and of constriction of the first cleavage upon the arrangement of organ bases, is not to be overlooked.

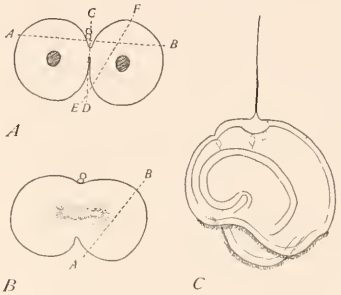


FIG. 5.

(4) The egg was cut along *EF* (5*A*). From the left half a perfect but dwarf pilidium resulted. This is a case worth describing, because the egg neither rounded up, as is usually the case, nor divided into two unequal halves, but soon after the cut surface was closed, the right half gradually increased in size accompanied by the decrease of the left half, and thus the egg was divided into two equal blastomeres.

SERIES E.

Development of the Blastomeres Isolated at the Two-cell Stage.

The blastomeres of eight eggs were separated at the two-cell stage when the cytoplasmic bridge had disappeared and the blastomeres had assumed a spherical form. From every blastomere a normal pilidium was developed. Some were about half the normal size, while some, for reasons that I cannot explain, were very

much smaller. It is remarkable that the development of the isolated blastomeres differs very widely from that of fragments of the period preceding. From a comparison with Series D it seems likely that the result may be different according to the period of cutting — *i. e.*, before or after the disappearance of the cytoplasmic bridge (cf. Series D, eggs *B* and *C*) and the pressing together of the blastomeres. In any event, further careful examination of the egg of this stage with reference to the question of egg specification is very desirable.

Conclusion.

From the above series of experiments we have seen that, if the cutting-off of a portion of cytoplasm is done before maturation, it only slightly affects the normal development; but if the operation is done at the first maturation stage the formation of the organs of the pilidia is considerably affected, and still more if it is done at the time of fusion of the germ-nuclei. We cannot, therefore, escape the conclusion that there must take place some progressive changes in the general make-up of the egg during the period extending from the time of dissolution of the germinal vesicle to the fusion of the germ nuclei. This period falls into two subdivisions, the first extending from the fading of the germinal vesicle to the metaphase of the first polar mitosis, and the second from the growing period of the sperm-nucleus to its conjugation with the egg-nucleus. The importance of these two periods has been correctly perceived by Delage (1901), Fischel (1903) and recently by Professor Wilson (1903). It is hardly necessary to state here that, although these two periods can be artificially separated by delaying fertilization, yet, under natural conditions, there is no pause between them, as the spermatozoön enters the egg before its maturation.

Let us next see what visible changes take place in the egg cytoplasm as the result of the above two acts, *i. e.*, the disappearance of the germinal vesicle and the entrance of the spermatozoön in the matured cytoplasm. As soon as the germinal vesicle breaks up, the nuclear fluids flow out and may diffuse through the egg. At this time currents may be formed carrying the egg cytoplasm from one spot to another. Although

in the *Cerebratulus* egg we have so far no direct evidence to support the occurrence of the last phenomenon, yet my observations on sections clearly show that a segregation of egg material does actually takes place at this period, the yolk accumulating in the lower hemisphere, while the clear and more finely granular protoplasm collects especially at the top of the egg, where, in the iron hematoxylin-Congo-red preparations, it stands out beautifully stained blue in contrast with the red yolk. After the fading of the germinal vesicle the eggs not only become fecundable, but also acquire much more power of forming cytasters than they had before. It is hardly necessary to state that in many forms remarkable changes take place in the egg at or after the entrance of the spermatozoön. It may further be pointed out, however, that the part played by the spermatozoön in causing rearrangements of the egg-substance is of a subsidiary character, as is shown in the case of parthenogenesis.

To the question as to what degree of localization exists in the cytoplasm of the *Cerebratulus* egg before the fading of the germinal vesicle, I am not in a position to answer, and it is almost impossible to find direct evidence. But the results of my experiments harmonize with Boveri's view that there is at first only a simple promorphological condition such as polarity and bilaterality, which may give a basis, so to speak, for a more definite grouping of material arising at the time of the flowing-out of the nuclear fluids. Otherwise it is impossible to understand the sudden increase in the proportion of abnormal embryos arising from the fragments obtained subsequent to the fading of the germinal vesicle. In the eggs of some forms (*e. g. Myzostoma*) the segregation of material has in a measure taken place long before maturation. In such cases the horizontal section of the egg before the disappearance of the germinal vesicle will, I think, produce a defective embryo. My observations on the later periods render it probable that at this time there must be a certain number of predetermined regions more or less firmly fixed, though this is not so clearly shown as in the ctenophore egg. It is extremely desirable to carry out careful studies with respect to the egg specification prior to the first cleavage in other forms.

So far as I could ascertain (up to the eight-cell stage), the cleav-

age of egg fragments was perfectly normal in Series A, B and C. It is especially noteworthy that the nucleated pieces of the fertilized eggs cleave exactly like a normal egg. Whatever amount of cytoplasm be cut off, the nucleated piece always divides at first into two and then into four equal blastomeres. Since these fragments so often gave rise to defective embryos, it is probable, not only that the two and four blastomeres differ from each other, but also that they do not correspond with those in the normal case. The same is true in the eight-cell stage, and so on. From this it seems further probable that cleavage of an egg takes its normal course irrespective of the localization of embryonic regions within. The cleavage pattern is stamped on the cytoplasm; but the end-result is governed by a different set of factors. The cleavage factors seem, therefore, to differ in this case from the morphogenic ones. "Though there is often a close and constant connection in the normal development between the process of cleavage and that of localization and differentiation, this connection is not a necessary relation" (Wilson, 1903[1]).

It is rather striking that, if the operation is done before the completion of the first cleavage, most of the egg fragments give rise to defective larvæ, while all of the isolated blastomeres at the two-cell stage develop into perfect pilidia (though the result is not conclusive, since my cases were too few). It is easy to conceive, however, that natural cleavage comes into operation in a quite different way from the artificial section, and it is probable that by the natural cleavage all the organ bases are equally distributed into two blastomeres which would be very unlikely after an artificial section.

Summary.

1. Before the germinal vesicle fades, there is no evidence of definite specification in the egg regions.

2. Dissolution of the germinal vesicle initiates the establishment of the germinal localization.

3. In the period between the entrance of the spermatozoön and the fusion of the germ nuclei, the localization becomes more definite.

4. The basis of the apical organ is not at the animal pole, but

[1] Wilson, *l. c.*, p. 439.

somewhere above the equator as a broad zone. The bases of the ciliated lobes and the gut lie mainly in the vegetative hemisphere.

5. The cleavage (up to eight-cell stage) is normal in an egg fragment obtained from the unsegmented egg, whatever be the amount of cytoplasm cut off, or at whatever period. It is probable that the cleavage factors do not here necessarily coincide with the morphogenic ones.

Zoölogical Laboratory, Columbia University,
October 29, 1903.

SOME OBSERVATIONS AND CONSIDERATIONS UPON THE MATURATION PHENOMENA OF THE GERM CELLS.[1]

THOS. H. MONTGOMERY, JR.

In a series of studies on the spermatogenesis of the Hemiptera, of *Peripatus*[3] and of the Amphibia,[4] I have endeavored to prove the following points:

1. That the chromosomes retain their individuality from generation to generation to that extent that a chromosome of one generation is not a new formation, however much its chemical substance has became changed by metabolism, but represents at least a part of a particular chromosome of the preceding generation.

2. That the first maturation mitosis in the species studied results in the separation from each other of entire univalent chromosomes, and hence is a transverse or reduction division; while the second maturation mitosis results in the longitudinal splitting of univalent chromosomes, and therefore is an equational division.

3. That the so-called "reduction in number" of the chromosomes is effected before the maturation divisions, by a pairing (conjugation) in the early growth period (synapsis stage) of the spermatocytes, of univalent chromosomes of like volume, each such composite chromosome then being bivalent with relation to the number in the spermatogonia.

4. That this conjugation of univalent chromosomes in the synapsis stage, is a conjugation of paternal with maternal chromo-

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 54.

[2] "The Spermatogenesis in Pentatoma up to the Formation of the Spermatid," *Zoolog. Jahrb.*, 12, 1898; "Chromatin Reduction in the Hemiptera: a Correction," *Zoolog. Anz.*, 22, 1899; "A Study of the Chromosomes of the Germ Cells of Metazoa," *Trans. Amer. Phil. Soc.*, 20, 1901; "Further Studies on the Chromosomes of the Hemiptera heteroptera," *Proc. Acad. Nat. Sci.*, Philadelphia, 1901.

[3] "The Spermatogenesis of Peripatus (Peripatopsis) balfouri up to the Formation of the Spermatid," *Zoolog. Jahrb.*, 14, 1900.

[4] "The Heterotypic Maturation Mitosis in Amphibia and its General Significance," BIOL. BULL., 4, 1903.

somes; and hence that the first maturation mitosis separates paternal from maternal chromosomes.

The third and fourth of these conclusions were new; the second has been a matter of much controversy, while the first has a considerable number of cytologists in its support. If these results can be generally established they will give a coherent basis for understanding the part played by the chromosomes in heredity, taken in conjunction with the important conclusion of Van Beneden[1] that the male pronucleus has a number of chromosomes equal to that in the female pronucleus; and of Henking[2] and O. Hertwig[3] that the maturation phenomena correspond very closely in the ovogenesis and spermatogenesis of the same species.

It is not my purpose here to go over the whole controversy and discuss in full the opposing views, for that has been done in my preceding papers, but rather to draw attention to a few of the more important results, and to add some new observations.

1. The First Maturation Mitosis in Amphibia.

Janssens and Dumez[4] have very recently reëxamined the spermatogenesis of Amphibia, particularly with regard to my results on *Plethodon* and *Desmognathus*, and decide that my position is both untenable and unproved: that the heterotypic mitosis is an equational division, and not a separation of entire univalent chromosomes as I had maintained. They state (p. 433): "On est tout étonné de ne trouver dans le texte de Thos. H. Montgomery *aucun argument* pour cette thèse." They do not mention my strongest argument at all, namely that the space enclosed by the heterotypic chromosome is a space separating two whole univalent chromosomes, and not a longitudinal split between two halves of a single chromosome, because this space is largest in the earliest stages, and not, as one would expect if it

[1] "Recherches sur la maturation de l'oeuf et la fécondation," *Arch. Biol.*, 5, 1883.

[2] "Ueber Spermatogenesis und deren Beziehungen zur Eientwicklung bei Pyrrhocoris apterus M.," *Zeit. wiss. Zool.*, 51, 1890.

[3] "Vergleich der Ei- und Samenbildung bei Nematoden," *Arch. mikr. Anat.* 36, 1890.

[4] "L'Elément nucléinien pendant les cinèses de maturation des spermatocytes chez Batrachoseps attenuatus et Pletodon cinereus," *La Cellule*, 20, 1903.

were a longitudinal split, smallest at those periods. In other words, I showed that at the earliest stage when the chromosomes can be distinguished, this space is largest, while the true longitudinal split is found in the axis of each arm of a heterotypic chromosome. They add: "L'auteur continue et, *sans en donner* la *moindre preuve* cette fois, que *les deux branches* de *l'anse s'enroulent l'une autour de l'autre* pour constituer les dyades enroulées, qui, d'après tous les auteurs, se trouvent dans les spermatocytes I avant la mise au fuseau des chromosomes." It is only necessary to rejoin that the comparison of the chromosomes as shown in my consecutive figures is sufficient proof. But when they say that my figures "sont fort schématisées," I simply answer that is not true, and that all were made with great care with the use of a camera lucida.

Janssens and Dumez do, however, bring up one good criticism. They note quite correctly that in the spermatogonia the chromosomes are of unequal lengths, while the two halves of a bivalent heterotypic chromosome are always of equal length. And they reason that if my view were correct that one of the heterotypic chromosomes is formed by the pairing of two univalent chromosomes, that I would have to demonstrate how two such conjugating chromosomes are always of the same length. For they argue that if the heterotypic chromosome were formed by a longitudinal splitting, such a splitting would fully explain the length equality of the two arms of the chromosome.

This is a good criticism, but I find it answered by a study of the relative volumes of the chromosomes in the equatorial plate stage of the spermatogonia. In every case where the pole view shows all the chromosomes lying in one plane, we can determine by carefully drawing them that there are just 12 pairs of chromosomes present, the two of each pair being of equal volume and frequently of similar form. One will find many cases where the pole view of a chromosome plate does not show this distinctly, but that is only when the chromosomes are irregularly arranged, and when their long axes do not lie in the plane of the equator.

A series of figures demonstrate this. Fig. 1 is the only case where all 24 chromosomes were seen in their entirety on pole

view; in Figs. 2–5 only such chromosomes were drawn as could be sent in their whole length; and in none of the figures were any chromosomes omitted that could be seen in their entirety. Two chromosomes of a corresponding volume are marked with the same letter, one in capitals and other in lower case, as, *e. g.*, *A* and *a*. All these cases were drawn, after numerous preliminary sketches, as accurately as possible with the camera lucida, and then those which seemed to correspond were lettered alike. Thus in Fig. 1, the large chromosomes *A* and *a* are alike, but differ in volume from all others; *B* and *b* are markedly alike, *C* and *c*, and all appear clearly paired. Sometimes, as in the case of *G*, *g*, *H*, *h*, the two of a pair appear dissimilar in the drawings, but this is simply because the curvature of one lies in a different plane from that of the other. Thus in Fig. 3 are shown also all 24 chromosomes, but the two marked *x* and *y* were seen so obliquely that it could not be determined whether they were alike. Fig. 6 shows a lateral view of a spermatogonic spindle, showing also a similar pairing of some of the chromosomes; and Fig. 7 shows the same phenomenon in an oblique pole view of a portion of one plate of daughter chromosomes of an early spermatogonic anaphase.

An examination of these Figs. 1–7, shows that the chromosomes are paired according to their volumes, that the two of a pair are very frequently of the same form; and that in most of the cases the two of a pair lie in the spindle close together. The only interpretation for this last condition is that corresponding chromosomes must have been arranged close together in the continuous chromatin spirem of the prophase, so that in the spirem *A* would be next to *a*, *B* to *b*, and so on. But on a study of nuclei in the late prophase (loose spirem) stage, I was not able to determine this positively, for until the chromosomes have shortened to their definitive forms, they are so irregular and twisted that it is practically impossible to determine their exact lengths on sections; probably crush preparations (such as those employed by Sutton) could be used with advantage here. At least there is no doubt of a continuous linin spirem in the spermatogonic prophase and of the arrangement of the chromosomes in a chain along this thread. And it is probable that

similar chromosomes are contiguous in this spirem, since in the metakinesis like chromosomes usually lie near each other; and, as Fig. 7 shows, they retain their contiguity even in the daughter cells.

Therefore we find, what Janssens and Dumez insisted that I should demonstrate, twelve pairs of chromosomes of the same length in the equatorial plate of the spermatogonia.

Now for the proof that such chromosomes unite into pairs in the spermatocytes, an explanation which, according to the Belgian cytologists, "est absolument fantastique et demanderait à être rigoureusement démontrée." On none of my preparations were there stages between the early anaphase of the last spermatogonic mitosis and the synapsis stage of the growth period. In the synapsis stage in *Desmognathus* the long and slender chromosomes are so intricately coiled together that it is impossible in sections to determine their exact relations. The cell has a distinct polarity, the nucleus at one end, at the opposite the greatest mass of cytoplasm containing the idiozome body. When the chromosomes begin to separate sufficiently for their boundaries to be distinguished, each appears as a loop or U with its ends at that part of the nucleus nearest the idiozome body. This is shown in Fig. 8, where only five of the loops are drawn in their entirety. On a transverse section of such a stage (Fig. 9) one finds 24 cross-cut portions of loop; every two of these portions correspond to the two arms of one of the U-shaped loop of Fig. 8. Therefore there are U-shaped chromosomes, with a particular arrangement, to the number of 12; hence they must be bivalent with regard to the 24 chromosomes of the spermatogonia. At this early stage (Fig. 8) there is no sign that the space enclosed by such a loop has arisen by a longitudinal splitting of a chromosome, for in fact the characteristic shape of the loops is the same now as at later stages (Figs. 10, 11). Now this was the main basis of my argument before — an argument that Janssens and Dumez ignore: that if this were a longitudinal split, we should find its commencement in such an early stage.

The true longitudinal split commences in the stage of Fig. 8, is very prominent in that of Figs. 9, 10; this is a clear splitting of each chromatin granule of the arms of each bivalent

chromosome, but the width of this split never becomes wider than that shown in Fig. 11. Janssens and Dumez, as all the workers on amphibian spermatogenesis before them, have entirely overlooked this split of each arm of a bivalent chromosome. With material stained in iron hæmatoxyline, and sufficiently destained, this split, though narrow, is perfectly distinct.

There is a point brought up by Janssens and Dumez to which attention must be drawn. They maintain (1) that there is no regular occurrence of a band of linin (that marked *k* in the figures of my previous paper), joining the two univalent arms composing a U, and placed at the bend of the U; and (2) that the linin spirem appears continuous and not broken into as many segments as there are U-shaped loops. A reëxamination shows me that they are right in regard to there being here a continuous linin spirem. And this is exactly what I gave especial study to proving to be the case in the corresponding stages of *Peripatus*. But I must maintain against these writers, that in *Plethodon* and *Desmognathus*, as in *Peripatus*, there is at no stage in the spermatocytes a continuous chromatin spirem. Sometimes one arm of a U-shaped chromosome seems to be continuous with the end of an arm of another, as in Fig. 11 (and Fig. 5 of my preceding paper). But this is unusual, and generally one finds, as in Figs. 8, 10 and 11, that the ends of the U-shaped chromatin loops are connected with the ends of other loops only by linin. Hence the boundaries of the bivalent chromosomes are perfectly distinguishable in most cases. As to the first point, I admit that the U-shaped loop, which I regard as composed of two univalent chromosomes attached at the bend of the U, does not show in all cases a band of linin at its bend; but it does nevertheless in many cases. However, on this point I did not place great insistence, as Janssens and Dumez maintain.

The U-shaped bivalent chromosomes of Fig. 11 shorten and condense into forms such as shown in Fig. 12. By the condensation of the chromatin the longitudinal split becomes hidden. This is not a remarkable phenomenon, as maintained by the Belgian writers; I and others have described it for frequent cases in arthropods. It reappears in the anaphase of the first maturation mitosis as a split along which the chromosomes

divide in the second mitosis. Finally the definitive shape is reached, as shown in Figs. 13 and 14. One peculiarity has often been described in heterotypic chromosomes: as they are placed in the spindle (Fig. 13), very frequently at the middle of each is one thickening, and this is more frequent than two thickenings. By comparison of a chromosome, such as that of Fig. 14, with earlier conditions, such as those of Fig. 12, it becomes evident that such a thickening corresponds to the separated ends of the U-shaped loop, which have finally came into close juxtaposition.

When we consider these points, we find two important facts: (1) that the twenty-four chromosomes are regularly paired in the spermatogonia, and that there the two of a pair lie close together; and (2) that there is no evidence that the space enclosed by any one of the twelve heterotypic chromosomes has been formed by a longitudinal splitting. In the spermatocytes there are twelve loops of the shape of a U or V. There is a longitudinal splitting, but along the long axis of each loop. The simple explanation of these facts is that in the spermatocytes, in the synapsis stage, there takes place the close conjugation of every two such chromosomes as were found in the spermatogonia; that two together constitute a U-shaped loop; and that therefore the first maturation division results in separating entire univalent chromosomes.

The difference of opinion between Janssens and Dumez and myself is more one of interpretation than of observation, though they did not notice the pairing of the chromosomes in the spermatogonia, nor yet the true longitudinal split. They frankly admit that by their interpretation the reduction in number of the chromosomes remains a mystery. They give no explanation of why there should be regularly disposed U-shaped loops. In assuming that a heterotypic chromosome has been formed by a longitudinal splitting, instead of by a junction of two univalent chromosomes, they contend for a kind of splitting very wide at the middle of the chromosome, but narrow at its ends; yet no such longitudinal splitting is known in any other case, and its difference is brought out sharply by comparison with the undoubted longitudinal splitting in the chromosomes of the later prophases of the spermatogonia. These heterotypic chromosomes

are different in form from others, just because they represent pairs of univalent chromosomes. If they arose by simple longitudinal splitting, why should they differ so in form from the chromosomes of the spermatogonia, or of the spermatocytes of the second order?

And here my thanks are due to Janssens and Dumez for this critique of my interpretation, because it induced me to study anew the amphibian spermatogenesis, and this reëxamination brought out the fact, strong in support of my position, that the chromosomes are regularly paired in the spermatogonia.

2. The Individuality of the Chromosomes.

By the idea of the maintenance of the chromosomal individuality we do not mean that a chromosome remains chemically unchanged from generation to generation (for after every mitosis a daughter chromosome grows to the size of a mother chromosome before it divides in the next mitosis), but that, despite great metabolic and structural changes, a chromosome of any generation is the descendant of a particular chromosome of the preceding generation, and is not a new formation. A chromosome of one generation represents a chromosome of a preceding, just as much as a cell of one generation represents a particular cell of a preceding generation. This idea was first propounded by Rabl,[1] and has steadily gained in support. The workers on ovogenesis have, for the most part, taken the position that it is not proved; but the reason there is simply the great duration of the growth period of the ovocytes, during a part of which chromosomal boundaries are not distinguishable. The students of spermatogenesis, on the other hand, are fairly unanimous in support of the view.

It is very important that this idea should be firmly established, and certain considerations would show it to be so. There is first the fact that from generation to generation the number of chromosomes remains the same, from the stage of the fertilized egg to that of the ovocyte or spermatocyte of the first order. Even the form of the chromosomes is maintained through these generations, as shown in the case of the cleavage of *Ascaris*. In the sperma-

[1] "Ueber Zelltheilung," *Morphol. Jahrb.*, 5, 1885.

togenesis of some Hemiptera there is no rest stage in the growth period of the spermatocytes, so that the chromosomes can be followed from the spermatogonia to the spermatids. In *Peripatus* this is but a short rest stage, and during it the boundaries of the chromosomes can be readily distinguished.

On the experimental side excellent evidence has been brought in support of this view, particularly by the study of abnormalities, by Boveri,[1] Zur Strassen,[2] Morgan,[3] and Herla.[4]

Evidence fully as strong as that from experimentaal study has been obtained by observations upon certain modified chromosomes of insects, which are:

3. The Heterochromosomes.

I offer this name to include those peculiarly modified chromosomes to which have been given the names "accessory chromosomes" by McClung,[5] "small chromosomes" by Paulmier[6] and "chromatin nuceoli" by myself. They have been described for the Hemiptera by Henking (*l. c.*), Paulmier, and myself; for the Orthoptera by Wilcox,[7] McClung, Sutton,[8] de Sinéty;[9] and for the spider by Miss Wallace.[10]

[1] "Zellen-Studien," *Zool. Jahrb.*, 1888; "Befruchtung," *Ergebn. Anat. Entw.*, 1891; "Ueber die Befruchtungs- und Entwickelungsfähigkeit kernloser Seeigel-Eier," *Arch. Entwickmech.*, 2, 1895; "Mehrpolige Mitosen als Mittel zur Analyse des Zellkerns," *Verh. Phys. Ges. Würzburg*, 35, 1902.

[2] "Ueber die Riesenbildung bei Ascaris-Eiern," *Arch. Entwickmech.*, 7, 1898.

[3] "The Fertilization of Non-nucleated Fragments of Echinoderm Eggs," *Arch. Entwickmech.*, 2, 1895.

[4] "Étude des Variations de la Mitose Chez l'Ascaride Mégalocéphale," *Arch. Biol.*, 13, 1893.

[5] "A Peculiar Nuclear Element in the Male Reproductive Cells of Insects," *Zool. Bull.*, 1899; "The Spermatocyte Divisions of the Acrididæ," *Bull. Univ. Kansas*, 1900; "Notes on the Accessory Chromosome," *Anat. Anz.*, 20, 1901; "The Accessory Chromosome — Sex Determinant?" Biol. Bull., 3, 1902; "The Spermatocyte Divisions of the Locustidæ," *Kansas Univ. Sci. Bull.*, 1, 1902.

[6] "The Spermatogenesis of Anasa tristis," *Journ. Morph.*, 15, 1899.

[7] "Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen," *Bull. Mus. Comp. Zool. Harvard*, 27, 1895.

[8] "The Spermatogonial Divisions of Brachystola magna," *Kansas Univ. Quarterly*, 9, 1900; "On the Morphology of the Chromosome Group in Brachystola magna," Biol. Bull., 4, 1902.

[9] "Recherches sur la Biologie et l'Anatomie des Phasmes," *La Cellule*, 19, 1901.

[10] "The Accessory Chromosome in the Spider," *Anat. Anz.*, 18, 1900.

These are chromosomes which preserve to great extent their compact form during the whole growth period of the spermatocytes, and during the rest stages of the spermatogonia, and retain throughout this whole period the deep staining characteristic of the other chromosomes only during the height of mitosis. Thanks to this peculiarity they can be followed with extreme certainty from generation to generation, even during rest stages; and so are splendid evidence for the thesis of the individuality of the chromosomes.

Now there are two kinds of these. In the Orthoptera there is an unpaired one in the spermatogonia, larger than the other chromosomes; in the Hemiptera they are paired in the spermatogonia, and usually smaller than the other chromosomes. Otherwise in their behavior they are very similar in these two groups of insects. To include both these kinds the name "heterochromosomes," as expressing a difference from the other chromosomes, can be advantageously applied; and this would include (1) the "accessory chromosomes" (unpaired in the spermatogonia), and (2) "the chromatin nucleoli" or "small chromosomes" (paired in the spermatogonia). McClung regards them as sex determinants; I have considered them to be chromosomes that are in the process of disappearance, in the evolution of a higher to a lower chromosomal number.

Now these can be followed from generation to generation without in the Hemiptera undergoing those profound changes which characterize the other chromosomes after a mitosis. In the Figs. 15–23 they are the chromosomes marked *N*, *n*; Figs. 16 and 17 show them in the spermatogonic and first spermatocytic mitoses of *Anasa*; Figs. 18, 19 for the same stages in *Corizus;* Figs. 20, 21 for *Trichopepla;* and Figs. 22, 23 for the spermatogonic monaster and late prophase of the first maturation respectively of *Protenor*. In all these cases they can be recognized in mitosis by their much smaller size.

Recently I have found them to occur in the same number and form in the ovogonia; Fig. 15 shows a pole view of the chromosomal plate in the ovogonium of *Anasa;* Fig. 16 a similar view of the spermatogonium of the same species, and the chromosomes marked *N* and *n* are found to correspond exactly.

Now for *Protenor* I found that in the spermatogonic chromosomal plate there are always exactly thirteen elements (Fig. 22); the two smallest of these are the heterochromosomes marked *N*, *n*; they are paired, and in the following synapsis stage conjugate to form the smallest bivalent chromosome *N*, *n* of Fig. 23; these two chromosomes are then quite similar to the heterochromosomes (chromatin nucleoli) of the other Hemiptera. But there is a large element in the spermatogonium (*X*, Fig. 22), unpaired there, and which does not conjugate with any other chromosome during the synapsis stage, but remains unpaired in the spermatocyte (*X*, Fig. 23). This element I called the "chromosome *x*." Now, as McClung has also pointed out, this chromosome behaves exactly as does an accessory chromosome in the Orthoptera, being unpaired in the spermatogonia, and not conjugating with any other chromosome during the synapsis stage. Therefore in *Protenor* occur both kinds of heterochromosomes, the small paired ones, *N* and *n*, and the large unpaired one, *X*. *N* and *n* are "chromatin nucleoli" according to my terminology, while *X* is an "accessory chromosome"; thus both kinds of heterochromosomes occur in the same cell, and their likenesses and differences were fully discribed by me for this species. Both are recognizable through the whole growth period of the spermatocytes by their compact form and deep staining; but only the small pair, *N* and *n*, can be recognized in the rest stage of the spermatogonia. In three other Hemiptera, *Alydus*, *Harmostes* and *Œdancala* I showed that in the spermatogonia occurred also an uneven number (thirteen) of chromosomes; but in these the odd chromosome does not maintain its compact form during the growth period of the spermatocyte, and so is not recognizable there; but in the first maturation mitosis it is immediately recognizable as the only chromosome that has not conjugated with another to form a bivalent one. Now such a chromosome of *Alydus*, *Harmostes* and *Œdancala* agrees with the chromosome *X* of *Protenor* in not pairing with another during the synapsis stage; but differs from it in behaving like the other chromosomes after a mitosis, *i. e.*, in losing its compact structure and strong affinity for chromatin stains.

Why should a heterochromosome be sometimes unpaired in the

spermatogonia, sometimes paired? When they are unpaired they are larger than the other chromosomes. This might imply that such an unpaired heterochromosome really represents two in close union, *i. e.*, is already bivalent in the spermatogonium. And this I think is a very probable explanation, in view, first, of the behavior of the unpaired chromosomes during the growth period of the spermatocyte in *Protenor* (fully described in my paper, "The Germ Cells of the Metazoa"); and, second, of the fact that such a chromosome sometimes shows a distinct constriction at its middle (shown for *Harmostes* in my paper, "Further Studies on the Chromosomes," etc.).

It is hoped that these considerations, in endeavoring to show the likenesses and differences of the two kinds of heterochromosomes, will bring more uniformity in the interpretation of these modified chromosomes, and that such chromosomes should always be taken into account in any discussion of the idea of the individuality of the chromosomes.

4. The Conjugation of the Chromosomes in the Synapsis Stage, and its Relation to the Reduction Division.

It is now determined for a considerable number of cases that in the early portion of the growth period of both ovocytes and spermatocytes there takes place the process known as the "reduction in number" of the chromosomes. Thus if there are twenty-four chromosomes in the spermatogonium, twelve are found in the maturation period before the first mitosis. This fact was first established by Boveri ("Zellen-Studien," *l. c.*) and by Brauer.[1] Really the name applied is a misnomer, for there is no loss of chromosomes, no true "reduction" in this number, but it is a conjugation of the chromosomes. Rückert[2] sought to explain it by stating that in the prophase of the first maturation division the chromatin spirem breaks into only half the normal number of segments. This, however, is inadequate as an explanation, for I showed in the "Spermatogenesis of Peripatus" that in the prophases of the first maturation mitosis there is a

[1] "Zur Kenntniss der Spermatogenese von Ascaris megalocephala," *Arch. Mikr. Anat.*, 42, 1893.

[2] "Zur Eireifung bei Copepoden," *Anat. Hefte*, 4, 1894.

continuous *linin* spirem, which probably does not break into segments until the metakinesis of the first maturation mitosis, but no continuous *chromatin* spirem. Hence it is not a question of chromosomes which were already contiguous remaining contiguous, but of chromosomes which were first separated (except for their linin connections) conjugating to form pairs during the synapsis stage. The criterion of the synapsis stage is such a pairing; and the term "conjugation" of the chromosomes represents the facts much better than the term "reduction in number."

The bivalent chromosomes so formed by conjugation, in the Hemiptera, *Peripatus* and Amphibia, become so placed in the equator of the spindle of the first maturation mitosis, that entire univalent chromosomes become separated. This is a true reduction division in the sense of Weismann. Each spermatocyte of the second order thus receives whole univalent chromosomes in one half the normal (somatic) number. While the majority of writers still hold that no such reduction division occurs, the idea being abhorrent to them, there are still a number who have furnished an array of facts that can be interpreted only as speaking for such an occurrence; thus Rückert, Häcker, Vom Rath, Korschelt, Henking, Paulmier, McClung, Sutton, Nichols, Griffin, Van Winiwarter, Lillie, Schockaert. But the arraying of names on the one side against those on the other is no argument in itself, and we may pass to the discussion of certain facts which harmonize completely with the occurrence of a reduction division, and remain unexplainable on any other basis.

First, it may be recalled that there is a divergence of opinion as to which of the two maturation mitoses is the reduction division, some holding that it is the first and others, the second. There is no good reason, save the probability that there would be expected uniformity in such important processes, that this division should always be in the first mitosis, or always in the second; for it is quite possible that there is a difference in this regard in different objects. In the discussion which follows we will assume it to be the first maturation mitosis since there occurs the reduction division in the objects specially studied by me.

Now I reached the conclusion ("A Study of the Chromosomes," etc.) that in the synapsis stage there is effected a conjugation of paternal with maternal chromosomes; under "paternal" understanding those derived from the spermatozoön, and under "maternal," those from the ovotid. The arguments for this were stated as follows:

1. In *Ascaris megalocephala univalens* there is the normal number of two chromosomes. The ovotid and spermatid have each only one. In the fertilized egg there is one derived from the spermatid, one from the ovotid; therefore the bivalent chomosome found in the maturation period of the spermatocyte or ovocyte must have been formed by the conjugation of a paternal with a maternal chromosome.

2. In the spermatogenesis of the Hemiptera there are usually two small heterochromosomes in the spermatogonia. These unite to form a bivalent one in the spermatocyte. They become separated from each other in the first maturation mitosis so that no spermatid receives more than one. Evidently then in the fertilized ovum since only one comes from the spermatid, the other must come from the ovitid. Therefore in the conjugation of the two in the synapsis, it is a conjugation of a paternal one with a maternal one. That was reasoned out without any knowledge of such chromosomes of the ovogenesis. Now I add Fig. 15, showing among the chromosomes of an ovogenic monaster stage the two small elements *N* and *n*, which are heterochromosomes of the same number and size as those found in the spermatogonium (Fig. 16, *N*, *n*). Therefore, there must be a conjugation in the ovogenetic synapsis stage, as well as in the spermatogenetic, of a paternal heterochromosome with a maternal one.

3. That besides the heterochromosomes, whenever there is recognizable in the spermatogonic chromosomal plate a pair of chromosomes notably different from the others in volume, there is always found in the first maturation mitosis a particular bivalent chromosome notably different in volume from the other ones, and so evidently formed in the synapsis by the conjugation of the two peculiar univalent ones of the spermatogonium. This bivalent chromosome is so placed in the first maturation spindle

that its two univalent elements pass to opposite cells, so the sperspermatid has never more than one of them. One of those in the spermatogonium must accordingly have come from the spermatid and one from the ovotid, which combined to give rise to that spermatogonium. And here, also, in the formation of such a bivalent chromosome in the synapsis there must be a union of a paternal with a maternal chromosome. No other explanation seems possible.

These conclusions, the numerical ratios of certain clearly distinguishable chromosomes in the spermatogonia, to certain equally distinguishable ones in the spermatocytes and spermatids, could be established for the heterochromosomes for some forty species of Hemiptera, and for other chromosomes in the cases of *Trichopepla semivittata*, *Protenor belfragei*, *Peliopelta abbreviata*, *Prionidus cristatus*, *Zaitha fluminea* and *Corizus lateralis*. To make this point clear a few figures of certain of these cases are given here again. In the spermatogonium of *Anasa* (Fig. 16), as well as in the ovogonium (Fig. 15), are recognizable among the 22 chromosomes, two very much smaller than the others (*N*, *n*, heterochromosomes), and two considerably larger (*A*, *a*); in the first spermatocyte there are eleven bivalent ones, six of which are shown in Fig. 17, and here we recognize again the chromosomes *N*, *n* and *A*, *a*. In *Corizus*, in the spermatogonium (Fig. 18) are two particularly small (*N*, *n*) and two particularly large chromosomes; and these recognizable again in the first maturation spindle (Fig. 19), Similarly in the case of *Trichopepla* (Figs. 20, 21). In the spermatogonium of *Protenor* (Fig. 22) are thirteen chromosomes; a particularly large one (*X*), two next in size (*K*, *k*), and two smallest (*N*, *n*). In the spermatocyte (Fig. 23) *X* is recognizable as being the largest; it is the odd chromosome and is not paired with any other. *N* and *n* are paired and so are *K* and *k*.

From these observations I concluded that probably in every case the chromosomes in the synapsis united to form bivalent pairs in such a way that the one of each pair was paternal and the other maternal; and I was able actually to demonstrate it in those cases where the differences in volume between the chromosomes were sufficient to allow them to be followed from genera-

tion to generation. And I could also prove that in all cases the two components of each bivalent chromosome always become separated from each other in the first maturation mitosis.

Following this came the paper of Sutton (*l. c.*), proving conclusively that in the spermatogonium of *Brachystola* the chromosomes occur regularly in pairs of graduated sizes, the two of a pair being always of the same length; that in the synapsis stage bivalent chromosomes are produced by the conjugation of every two chromosomes of the same length; and that corresponding chromosomes became separated from each other in the reduction division (here the second maturation mitosis). So he concluded quite rightly that there are two series of chromosomes in the spermatogonium, a paternal series, *A*, *B*, *C* . . . *n*, and a maternal series, *a*, *b*, *c* . . . *n*, in which *A* corresponds to *a* in size and hereditary value, *B* to *b*, and so through the series. By *A* joining with *a* in the synapsis, like chromosomes conjugate; and by these separating from each other in the reduction divisions, it results that two chromosomes of like size are not found in the spermatid. Sutton was the first to demonstrate this for the whole series of chromosomes, and to argue that such a conjugation, together with the following reduction division, would operate so that no spermatid could receive two chromosomes of like hereditary value, but only one chromosome representing a particular value.

In the present paper I have shown that in *Plethodon* and *Desmognathus* also one may recognize the two corresponding series of chromosomes in the spermatogonium. An examination of *Ascaris megalocephala bivalens* shows the same relation. Pole views of the first cleavage spindle (Figs. 28–30) show each two larger (*A*, *a*) and two smaller chromosomes (*B*, *b*). The differences in size of the two pairs is not very great, but always recognizable when the chromosomes can be seen in their entirety. This is then evidently a case parallel to those described above: that of the larger pair (*A*, *a*) one is paternal and the other maternal, and that of the smaller pair (*B*, *b*) the same relation holds. Now the formation of the tetrads in the ovogenesis of this species has been described by Boveri as two equation (longitudinal) divisions of each bivalent chromosome; and Braur has reached the same result for the formation of the tetrads in the spermatogen-

esis. But in concluding this these writers do not give a satisfactory explanation either why or how univalent chromosomes unite to form bivalent ones, and so give no clue to a reason for the chromosomes being paired in the fertilized egg. This point can be settled only by a careful reëxamination of the changes in the early growth period, and here I shall simply call attention to certain appearances that speak for the first maturation mitosis in *Ascaris* being a reduction division.

In the fertilized egg are two pairs of chromosomes, *A*, *a* and *B*, *b* (Figs. 28–30), one pair being considerably larger than the other, and the two composing a pair sometimes differing somewhat in length but being approximately equal in volume. As Van Beneden first showed, two of these chromosomes come from the ovotid, and two from the spermatozoön. Fig. 27 shows a slightly earlier stage, the chromosomes in two groups, one group derived from the male pronucleus and the other from the female pronucleus. In the group to the right is a large and a small chromosome (*A*, *B*); in the group to the left also a large and small one (*a*, *b*). But *A* corresponds approximately to *a* in volume, and *B* to *b*. Therefore we may say that of the four chromosomes in the fertilized egg (Figs. 27–30) a small paternal one (from the male pronucleus) corresponds in volume to a small maternal one (from the female pronucleus), and a large paternal one to a large maternal one. In other words, of each pair of chromosomes in the fertilized egg, one chromosome is paternal and one maternal. Which two come from the male pronucleus, and which two from the female pronucleus, there is as yet no means of deciding, for the two pronuclei appear structurally alike. But to make my argument clear I will assume that *A* and *B* are paternal, and *a* and *b* maternal.

Now in the formation of the first polar body (Figs. 24, 25) we find the two well-known quadripartite chromosomes. There are two, not four; hence they are bivalent with regard to the normal number. In each bivalent chromosome (tetrad) we should expect then to find two univalent chromosomes. Both these figures (24, 25) were drawn with great care to get the exact proportions of the parts of the chromosomes; both represent the stage where one plate of chromosomes is passing into the polar body.

Now in each of these cases we notice in the polar body, as in the egg, a larger and a smaller bipartite chromosome. Thus in the polar body the larger dyad *A*, *A*, and the smaller *B*, *B*; in the egg the larger dyad *a*, *a*, and the smaller, *b*, *b*. As far as I can determine this relation appears to be constant: one large and one small dyad in the polar body as well as in the egg; and not two smaller (or larger) dyads in the polar body and two larger (or smaller) dyads in the egg. Now from what we have found to be the case in other objects, I would judge *A*, *A* to be an entire univalent chromosome that had been paired previously with the entire univalent chromosome *a*, *a*; and that a similar relation holds between *B*, *B* and *b*, *b*. This would then be a reduction division, separating entire univalent chromosomes. In favor of this is the fact that *A*, *A* is approximately similar in volume to *a*, *a*, and *B*, *B* to *b*, *b*; and we have learned that chromosomes of similar volumes conjugate in synapsis. Two dyads are left in the egg, *a*, *a* and *b*, *b*, each of which could be regarded as a longitudinally split univalent chromosome. In the formation of the second pole body (Fig. 26) the two parts of each dyad separate from each other, and this would be an equational division. There are then left in the egg the two chromosomes *a* and *b*, which differ markedly in volume. And this is in exact accord with the fact shown in Fig. 27, that from each pronucleus comes one large and one small chromosome.

This interpretation would bring *Ascaris* into close agreement with the other objects discussed in this paper: it explains why there are two large and two small chromosomes in the fertilized egg; why each pronucleus has one large and one small chromosome; finally, why the two bivalent chromosomes of the first maturation mitosis differ in volume. The idea that each such bivalent chromosome has been formed by a double longitudinal splitting, hence that both divisions are equational, gives no explanation for any of these phenomena, nor yet explains how or why bivalent chromosomes should be formed. The onus no longer rests upon us to prove the occurrence of a reduction division; but upon those of the other school to prove that a bivalent chromosome represents one chromosome that has undergone a double longitudinal division, and to show that such an interpre-

tation is explanatory of the kind of phenomena that we have discussed.

5. Chromosomal Combinations and the Mendelian Ratio.

In his paper on "The Chromosomes in Heredity"[1] Sutton argues that the combination of paternal and maternal chromosomes in the fertilized egg would result in a Mendelian ratio. It will be recalled that Mendel[2] in his experiments on crossing varieties of *Pisum*, to determine the law of transmission o parental characters to the hybrid, found the crosses to result in the ratio 1 *D* : 2 *Dr* : 1 *r*, in which *D* represents the pure character of one parent, *r* the pure character of the other parent, and *Dr* represents the possession of both characters. In other words: out of four offspring resulting from such a cross, one would resemble the father, one the mother, and two combine the characters of both parents.

Sutton starts with the fact that there are two series of chromosomes in each fertilized egg, *A*, *B*, *C*, ... *n* and *a*, *b*, *c*, ... *n*, the first set of paternal origin (from the spermatozoön) and the second of maternal (from the ovotid). In these series *A* is the homologue of *a*, *B* of *b*, and so on. In the synapsis stage of the germ cells *A* would conjugate with *a*, *B* with *b*, and so on, so there would be formed the bivalent chromosomes *Aa*, *Bb*, *Cc*, ... *n*. In the reduction division *A* and *a* would pass to separate cells, and such would be the case with *B* and *b* and the remaining paired chromosomes.

Then Sutton takes the case where there are the two homologous chromosomes *A* and *a* in an ovogonium and *A* and *a* in a spermatogonium; in the maturation period there would be formed *Aa* in the ovocyte, and *Aa* in the spermatocyte; the ovotids would contain then either *A* or *a*, and the spermatids either *A* or *a*. There would then result in fertilization these combinations:

$$A\,♂ + A\,♀ = AA$$
$$A\,♂ + a\,♀ = Aa$$
$$a\,♂ + A\,♀ = aA$$
$$a\,♂ + a\,♀ = aa.$$

[1] Biolog. Bull., 4, 1903.

[2] "Versuche über Pflanzenhybriden," *Verh. nat. Ver. Brünn*, 4, 1865.

"Since the second and third of these are alike the result would be expressed be the formula $AA : 2Aa : aa$ which is the same as that given for any character in a Mendelian case."

But as a matter of fact this can be so only in a case where there are only two chromosomes in the fertilized egg. For let us take the case where the normal number of chromosomes is four; express by capital letters those chromosomes originally derived from the spermatozoön, and by small letters those derived from the ovotid; and assume that A is homologous to a, and B to b. Then the spermatogonium would have the chromosomes, A, a, B, b, and the ovogonium have also A, a, B, b. By the synapsis stage would be formed bivalent chromosomes Aa, Bb in the spermatocyte, and Aa, Bb in the ovocyte. The reduction division would separate A from a and B from b in both spermatogenesis and ovogenesis. The spermatids would contain then either A, B, or a, b, or A, b, or a, B; and the ovotids either A, B or a, b, or A, b, or a, B. In the fertilization of one of these ovotids by one of the spermatozoa, 16 different combinations are possible: A, B, A, B; A, B, a, b; A, B, A, b; A, B, a, B; a, b, A, B; a, b, a, b; a, b, A, b; a, b, a, B; A, b, A, B; A, b, a, b; A, b, A, b; A, b, a, B; a, B, A, B; a, B, a, b; a, B, A, b; a, B, a, B. But only one of these combinations is of purely paternal chromosomes, namely A, B, A, B; and only one of purely maternal, namely a, b, a, b. The other fourteen combinations show paternal together with maternal chromosomes (six cases where the paternal and maternal chromosomes are present in equal number, four cases where there are three paternal chromosomes to one maternal, and four cases where there are three maternal chromosomes to one paternal).

Hence the ratio is : $1P : 14PM : 1M$, where P stands for purely paternal chromosomes, M for purely maternal, and PM for combinations of paternal and maternal chromosomes. This is clearly not a Mendelian ratio of 1 : 2 : 1. And obviously the disparity would become greater with any increase in the number of chromosomes. According to Sutton's own computation, in forms which have 24 chromosomes, the number of possible combinations of these in the fertilized egg would be 16,777,216. That would give the ratio of $1P : 16{,}777{,}214PM : 1M$.

But though the combinations of paternal and maternal chromosomes in the fertilized egg do not support the Mendelian ratio for hybrids, I fully agree with Sutton that "the phenomena of germ-cell divisions and of heredity are seen to have the essential features, viz., purity of units (chromosomes, characters) and the independent transmission of the same."

UNIVERSITY OF TEXAS,
November 29, 1903.

Explanation of Figures.

Figs. 1–3. Pole views of spermatogonic monasters of *Plethodon cinereus*.

Fig. 4. Pole view of a spermatogonic monaster of *Diemyctilus viresceus*.

Figs. 5, 6. Pole and lateral views respectively of spermatogonic monasters of *Desmognathus fuscus*.

Fig. 7. Oblique pole view of one plate of daughter chromosomes, early spermatogonic anaphase of *Desmognathus fuscus*.

Fig. 8. Lateral view of a late synapsis (postsynapsis) stage of *Desmognathus fuscus;* four entire bivalent chromosomes shown, and half of another. Nuclear membrane not yet formed.

Fig. 9. Polar view of a spermatocytic nucleus at a slightly later stage in the same species.

Figs. 10–12. Successive prophases of the first maturation mitosis in *Desmognathus fuscus*.

Fig. 13. Lateral view of a spindle of the first maturation mitosis, showing three bivalent chromosomes in metakinesis; *Plethodon cinereus*.

Fig. 14. A heterotypic chromosome of *Plethodon cinereus* in its definitive form.

Fig. 15. *Anasa* (undetermined species from California), pole view of ovogonic monaster.

Fig. 16. *Anasa* sp., pole view of spermatogonic monaster.

Fig. 17. *Anasa* sp., lateral view of first maturation mitosis, showing six of the eleven bivalent chromosomes.

Fig. 18. *Corizus alternatus*, pole view of the spermatogonic monaster.

Fig. 19. *Corizus alternatus*, lateral view of first maturation mitosis, showing five of the bivalent chromosomes.

Fig. 20. *Trichpepla semivittata*, pole view of spermatogonic monaster.

Fig. 21. *Trichpepla semivittata*, lateral view of first maturation mitosis, showing all the bivalent chromosomes.

Fig. 22. *Protenor belfragei*, pole view of spermatogonic monaster.

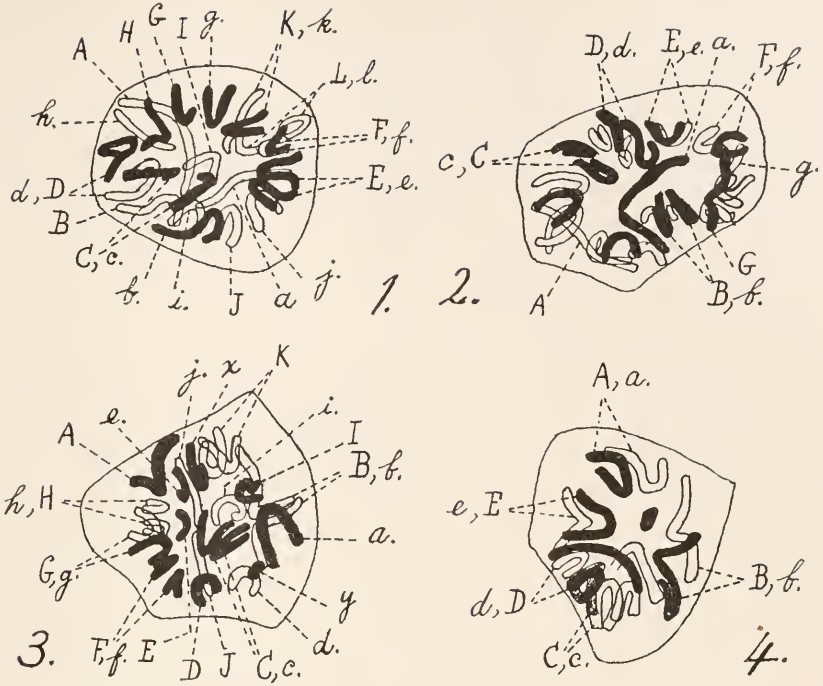
Fig. 23. *Protenor belfragei*, late prophase of the first maturation mitosis.

Figs. 24, 25. *Ascaris megalocephala bivalens*, formation of the first polar body (first maturation mitosis), the spindle seen very obliquely in Fig. 25.

Fig. 26. *Idem*, formation of second polar body.

Fig. 27. *Idem*, lateral view of the first cleavage spindle showing the two groups of chromosomes.

Fig. 28–30. *Idem*, pole views of the chromosomes in the first cleavage spindle.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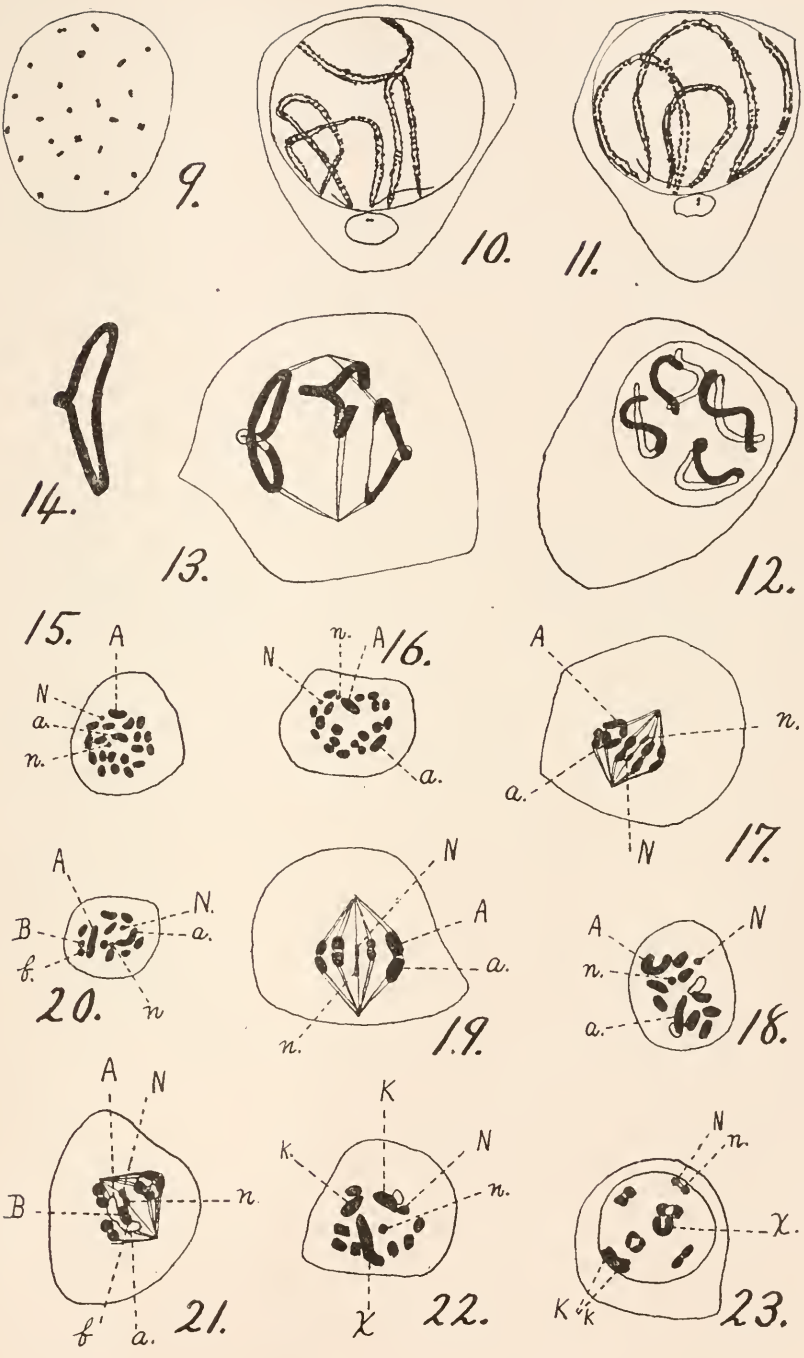
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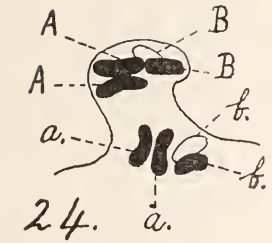

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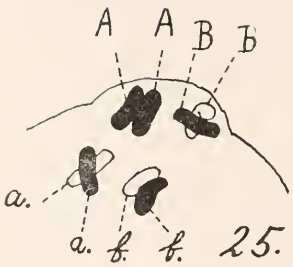

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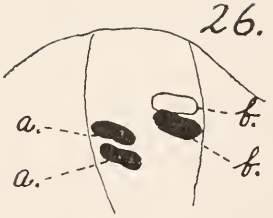

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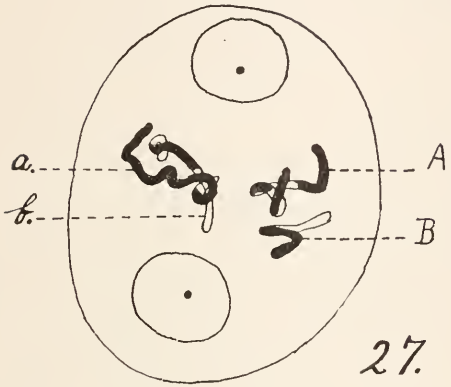

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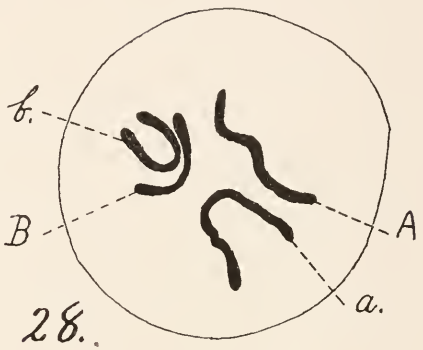

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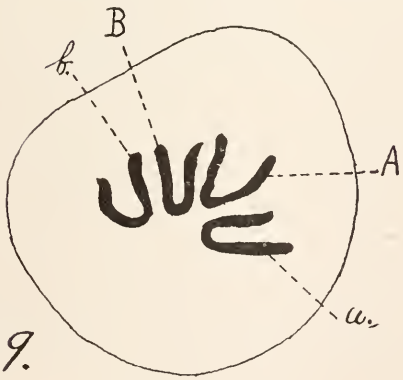

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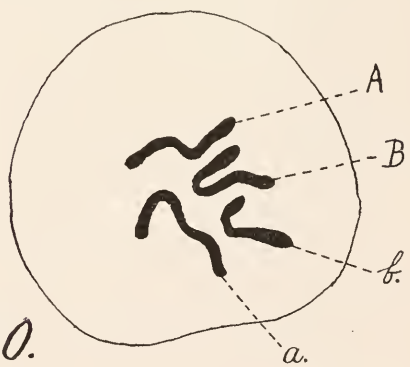

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BIOLOGICAL BULLETIN

NOTES ON REGENERATION.

T. H. MORGAN.

During the past summer I made at Woods Holl a number of observations and experiments on the regeneration of several animal forms. The results are here brought together, although they have little more in common than that they all deal with problems of regeneration.

The Limitation of the Regenerative Power of Dendrocœlum lacteum.

The fresh-water planarians show such remarkable powers of regeneration that it is surprising to find in one of them, *Dendrocœlum lacteum*, that this power is much reduced. The question at once arises whether we can discover anything peculiar in the relation of this planarian to its surroundings, or in its internal structure that will give a clue to its exceptional behavior.

There is nothing in its habitat to suggest that it has lost, or has never acquired to the same degree, the power of regeneration possessed by other planarians. In the pond at Falmouth where I collected this species there were also present, sticking to the under surfaces of the same stones, both *Planaria maculata* and *Phagocata gracilis*. If *Dendrocœlum* is not as subject to injury as are the other two species, and if, therefore, it does not need the same regenerative power, it is remarkable that *Dendrocœlum* should be so uncommon in comparison with the other two forms. If it is subject to greater injury, then it has not acquired the power to meet the situation as have the other species. Considerations of this kind do not have, I believe, any real bearing on the question of whether an organism has or has not acquired the power to regenerate, although some biologists lay great stress on this sort of speculation. The limitations in the power of regeneration of *Dendrocœlum* are peculiar. Lillie found that when only

the anterior end of the worm is cut off a new anterior end is regenerated. This power to produce a new head was found to extend back to about one-third of the length of the worm, *i. e.*, to a region just in front of the pharynx. Behind this level the posterior piece fails to regenerate a head at its anterior end.

On the other hand, the anterior pieces regenerate a new posterior end from any level, with the possible exception of the immediate region of the head itself; but the latter point has not yet been sufficiently examined in this species. It appears a remarkable fact that this planarian should have such extensive powers of regenerating posteriorly, and such limited powers of regenerating anteriorly, especially since, as far as we know, the same cells produce either a head or a tail according to which end is exposed; but this has not been definitely determined, and would be almost impossible to determine with absolute certainty. Eugen Schultz has also studied the regeneration of *Dendrocœlum lacteum* of Europe[1] and finds that posterior pieces do sometimes regenerate a head, although the regeneration is very slow, and it may appear that Lillie did not keep his pieces a sufficiently long time for the regeneration to take place. He states, in fact, that most of the posterior pieces died after five or six days. Schultz believes that these posterior pieces have potentially the power to regenerate, but that sometimes the piece closes in such a way that the formation of new tissue is prevented, as I have found to occur occasionally in *Bipalium*. Lillie, on the other hand, tries to account for the lack of power of posterior pieces to form a head by means of the following hypothesis. He suggests that the regeneration from the posterior cut surface at all levels is due, in some unexplained way, to the presence "of the brain and anterior part of the nervous system in the anterior piece." Conversely the absence of these structures in posterior pieces is supposed to account for the lack of regeneration from the anterior cut surface. A simple experiment would have shown the untenability of this point of view. If the head end is cut off just in front of the pharynx so that the brain and the anterior part of the nervous system are removed, and then the tail end of the middle

[1] It has been assumed that the European *Dendrocœlum lacteum* and the American form or forms are identical, but I think this question will bear further examination.

piece is also removed, it will be found that the middle piece without regenerating a new head will still regenerate a new tail. This shows conclusively that Lillie's supposition in regard to posterior regeneration is erroneous. The remainder of his argument, which rests on this assumption, also falls, I believe, in the light of this fact.

The great mortality that Lillie observed in the posterior pieces is due largely, at least in my experiments in which the same thing was observed, to the temperature being too high, or possibly to exposure to light. If the pieces are kept cooler (by surrounding the dishes by the cool, running salt water of the laboratory) the mortality is much reduced, and instead of dying after six days, as in Lillie's experiment, I have kept short posterior pieces for several weeks. It is only by keeping such pieces for a long time that one can fairly test their powers of regeneration.

Schultz states that he cut *Dendrocœlum* in two either between the pharynx and the reproductive region or else in front of the pharynx. In the former case he found that the posterior pieces regenerated an anterior end very slowly, and he found it more profitable in studying the regeneration of the head to use those posterior pieces that had been cut off in front of the pharynx. He found that the regeneration of the anterior end often failed to take place, and he attributes this to fusion of the sides of the cut surfaces, as I had found to occur not infrequently in *Bipalium*. Whether this is the whole of the question remains to be seen. In a marine polyclad, *Leptoplana*, Schultz found that posterior pieces, no matter at what level they have been removed, fail to regenerate an anterior end, even when only a small piece of the head is cut off. Yet regeneration from a posterior cut surface takes place at all levels. Schultz attributes the lack of regeneration at the anterior end either to the closing over of the "growing point" by the coming together of the old tissue from the sides, or to the muscles from the sides uniting and thus preventing further growth. Both factors he thinks may enter into the result. This point could be tested, I think, by making the cuts so that there is left a pointed anterior end, when regeneration should occur, if Schultz's view is correct. From an experiment of this sort that I have carried out on *Dendrocœlum* I think it

probable that in *Leptoplana* also no better regeneration would occur, even at a pointed end,[1] and if this proves to be the case Schultz's explanation is insufficient.[2]

In my experiments I first examined whether the form of the cut surface at the anterior end had anything to do with the lack of regeneration, for it was possible here, as in the case of *Bipalium*, that the cross-cut surface closed in such a way that subsequent regeneration was prevented. By changing the form of the cut surface this difficulty should be eliminated. Posterior pieces were cut off through the region of the pharynx and also behind the pharynx. The anterior ends of some of these pieces were very oblique; others were pointed in the middle, *i. e.*, they were cut off by two oblique cuts meeting in the middle line. In the latter case especially it is impossible that the muscles from the sides could close the anterior cut surface.[3] These pieces were kept alive for two or three weeks, and although it could be seen that there was a little new tissue at the anterior cut surface, yet no further regeneration occurred after the first ten days or thereabouts, and there is no indication that regeneration would have gone any further if the pieces had been kept alive for a greater length of time.

Sections of these pieces were made. The results will be given below.

In two other series each worm was cut into three pieces. The head pieces extended to the middle of the region in front of the pharynx. These pieces should be capable of regenerating at the

[1] Loeb says that *Thysanozoon* regenerates a new head, but he did not determine whether a new brain is formed. Monti also obtained regeneration in this form *and also in Leptoplana*, except when cut far posteriorly. Lang also records regeneration in marine polyclads.

[2] Schultz states in the opening of his paper that I carried out my experiments without making sections of the planarians, and he intimates that had I done so I would not have reached certain conclusions in regard to the growth of the new part. How Schultz obtained this information it would be interesting to know. Probably he based his generalization on the absence in my earlier papers of reference to histological details with which I was not then especially concerned. As a matter of fact I had made and studied many sections. My students also were at work on the minute anatomy, and one of them published a complete account of the histological changes taking place during regeneration before Schultz's paper appeared.

[3] Whether union of the dorsal and ventral muscles might close these pieces I have not here considered.

posterior end. The middle pieces included the next portion of the worm, and extended to the region of the reproductive pore. These pieces should be capable of regenerating a head at their anterior ends and a tail at the posterior ends. The third pieces were the tail pieces and included the rest of the worm. These pieces should be incapable of regenerating a head at the anterior end. The pieces were preserved at intervals of 1, 2, 3, 4, 5, 6 days, killed, embedded, stained and examined with immersion lenses.

A study of the sections shows that the changes taking place at the anterior end of the tail-pieces appear to be similar in all respects to those that occur at anterior or posterior surfaces at which regeneration of the missing part takes place. There is nothing in the sections to show why the regeneration should continue in the one case and not in the other, and it is difficult to believe from the evidence of the sections that anterior regeneration from the tail-pieces would not in time be accomplished, yet after three weeks there was no sign of further regeneration and I am forced to conclude with Lillie that in the form of *Dendrocœlum* found at Falmouth regeneration does not, ordinarily at least, occur behind the level of the pharynx. Sections through tail-pieces, cut off behind the pharynx and kept for nearly three weeks, show that the formation of new tissue has not gone much beyond that of the first six days, and that a new head has not been produced. Sections of the oblique, and of the pointed tail-pieces give exactly the same results.

Several writers seem inclined to account for the lack of regeneration in certain planarians, and especially from the posterior region of the body, as due to the absence or small size of the nerve cords in these regions. With this view I do not agree. Lillie has used *Dendrocœlum* as a case in point. Sections of this worm show, however, that the cords in the more posterior regions are as well developed, judging from their size, as they are in *Planaria maculata*.

Regeneration in Pycnogonids.

In 1895 Loeb published some observations that he had made on the regeneration of one of the Pycnogonida, *Phoxichilidium*

maxillare. He cut the animal in two between the second and third pairs of legs, and found in two cases that after a time a new part suddenly appeared, presumably after a moult. This new part that regenerated at the posterior end of the anterior piece Loeb speaks of as a body, and points out that this is the first case observed in the arthropods in which new body segments have been seen to regenerate. I have repeated this experiment during two summers, for it did not appear to me beyond dispute that the new part that had been observed was necessarily a body, since no satisfactory evidence that it was such is furnished by Loeb's paper. Although sections of the new part were, apparently, made, no posterior opening of the digestive tract was found, no ganglia are described as being present in the new body, nor do new legs appear to have been present at the sides as we should expect if this new part were really a body.

My first experiments were made in 1901 and, although a number of pycnogonids were kept for two months or longer, none of them regenerated at the posterior end. Since I had used large individuals it seemed not improbable that the lack of regeneration might have been connected with the maturity of the individuals. During the past summer I have repeated the experiment on a large scale, both with large and with small individuals; but although many of the pieces were kept for nearly two months no regeneration took place, with the possible exception of two instances that will be described.

In a number of cases the individuals were cut in two between the third and fourth pairs of legs, *i. e.*, nearer the posterior end than Loeb had cut them, for, from analogy with other cases, it seemed more probable that if the body could regenerate at all it would be more likely to do so the nearer the cut was made to the posterior end. Other individuals were cut in two between the second and third pairs of legs. In only one case did regeneration appear to take place, as shown in Fig. 1. Here the bases of the fourth pairs of legs bulge out as though they had been formed anew, and it seems possible that the rudimentary abdomen is also new, although it is also possible that a part at least of this structure had been left unintentionally when the cut was made. Sections show that the digestive tract opens at the

end of the abdomen. There is no trace of further regeneration within the stumps of the legs. At most, the bases of the legs and the abdomen, or part of the latter, have regenerated.

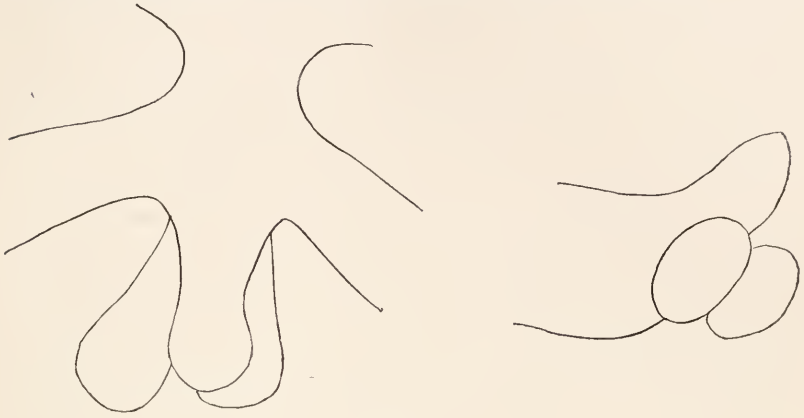


FIG. 1.

The second case is shown in Fig. 2, in which there is only a bulging out of the end of the body. The cut had been made in this case between the second and third pairs of legs. Sections of this individual do not show any indications of the development

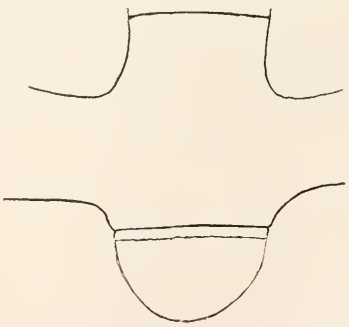


FIG. 2.

of legs or of a rudimentary abdomen in the new tissue of the bulging portion, and there is nothing to indicate that the development would ever have gone any further. The digestive tract ends blindly and is not connected with the ectoderm.

In looking over a large number of individuals I found a few cases in which a leg on one side was much smaller than its

opposite, and from this it seems probable that the original leg had been lost at the breaking joint at the base, and a new one had begun to regenerate. Moreover, I found one case in which the new leg was clearly a new structure, Fig. 3. The different segments had not yet been formed in their adult proportions and

FIG. 3.

the leg could not have been functional as yet. There is some resemblance between this leg and the newly regenerated part from the posterior end of the body that Loeb saw and figured. In fact this idea seems to have suggested itself to Loeb for he writes: "Das Vorhandensein eines ueberzähligen Segmentes könnte vermuthen lassen, dass das neugebildete Stück vielleicht im Laufe der Zeit sich zu einer Extremität entwickelt haben würde, dass es sich also um die Bildung eines Beines an Stelle des abgeschnittenen Rumpfstückes gehandelt habe, ein Fall, den ich als Heteromorphose bezeichnete. Allein Hoek führt an, dass bei Ammotheen das Abdomen nicht selten Spuren einer Segmentation zeigt." Thus in order to explain away the presence of too many segments in the new part Loeb has recourse to a condition found in another species — a mode of explanation that will scarcely recommend itself.

A somewhat fuller analysis of these two cases of Loeb's may not be unprofitable. If the new part is really a body, *i. e.*, thorax and abdomen, we should expect to find the digestive tract opening at the posterior end, but this does not appear to have been the case, for, Loeb says: "Der Darm setzte sich in den vorderen Theil des regenerirten Stückes fort. Im Uebrigen aber waren die Gewebe wenig differenzirt." It is to be remembered that the digestive tract also continues out into the legs in the pycnogonids as a blind sac. In the second place, while the three segments of his first example might be interpreted as representing the two remaining thoracic segments, and the rudimentary abdomen, yet in the other case five or six segments appear in the new part. It is this that led Loeb to suggest that the new part

might represent a leg; but he withdraws this interpretation at once as seen above. There is certainly no striking resemblance between the new part figured by Loeb and the abdomen of *Ammothea*. Finally, if the new part is a new thorax where are the legs?

In the light of these considerations we must wait until some one, favorably situated, has an opportunity to work over the subject with ample materials. Meanwhile it seems to me that so far as the evidence goes it is rather in favor of the view that the regeneration described by Loeb is a new leg and not a part that replaces the lost segments of the thorax and abdomen.

The Lack of Regeneration of the Pigment Spot in the Fin of Fundulus.

If a gold fish having a black band at the end of its tail be selected, and the end of the tail be cut off proximal to the band, a new band like the one removed reappears in the regenerated tail. The presence of black pigment at the cut surface from which the new part regenerates is clearly not necessary for the development of pigment in the new part. This result is all the

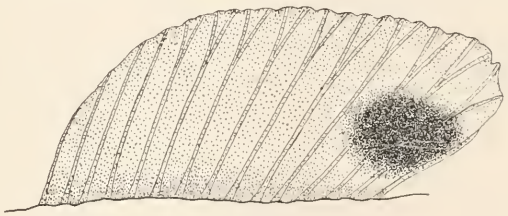


Fig. 4.

more curious since the occurrence of the pigment band is only an individual peculiarity. It seemed desirable to try the same experiment in a species in which a characteristic spot or a ring was present. The dorsal fin of the male of *Fundulus majalis* has a black spot in its posterior part, Fig. 4. The spot is not present in the female, and it appears, therefore, that this color marking belongs to the category of secondary sexual characters.

The posterior part of the fin was cut off by an oblique cut; the part removed containing all of the black spot. The lost part was slowly replaced, and in the course of two months the fin was completed, but the pigment spot did not come back, and there

was no evidence that it would have done so if the fish had been kept longer. Since the operation had been carried out during the height of the breeding season, it seemed possible that the spot might normally fade out later, but other fish, examined in September, showed the spot still present.

The results on *Fundulus* appear to be different from those on the gold fish, and it is not apparent why this difference should exist. The result does not seem to be connected in any way with the fact that the spot in *Fundulus* is a secondary sexual organ. The most plausible explanation that suggests itself is that in the tail of a gold fish that has a black tip there are cells throughout the tail that can develop pigment should they get into the terminal portions of the tail, while no such cells are present in *Fundulus*, or if present they fail to produce pigment in the new part. It may be that in *Fundulus* all the cells capable of producing pigment have been already carried into the pigment-spot itself, and hence when this spot is removed no cells capable of developing this pigment are present in the remaining part. Further work will be necessary to determine whether these suggestions have any value.

The Method of Closure of the Cut Ends of Tubularia.

The peculiar method of closure of the cut ends of *Tubularia* has attracted attention since it appears to be different from the closing observed in other forms. I have already discussed at some length this process [2] and shall not repeat here what has been already said, but since I have observed during the past summer certain processes that seem to throw some light on this question I shall briefly refer to them in this connection. Stevens has figured the closed end of a piece that had been cut through the hydranth-forming region at the time when the primoidium of the new hydranth had just been laid down, and when the red pigment lines, that indicate the appearance of the new hydranth, were present. Over the closed end the red lines radiate to the center of the bounding membrane. It seemed to me that a further examination of pieces that closed in this way might throw some light on the process in general. Pieces were cut off and

[1] Morgan, *Roux's Archiv*, XIV., 1902.

[2] "Regeneration," 1901, p. 69.

kept about twenty-four hours when the primoidia of the new tentacles had begun to appear. At this time I cut the ends squarely off, the plane of section lying across the middle of the new proximal tentacles. To my surprise the cut ends now closed in a very different way from that of ordinary cross-cut pieces. The whole wall contracted from the perisarc and the cut edges were brought together almost at once, and subsequently fused, often showing the radiating lines described by Stevens over the new end. It was perfectly clear that the result was due to a contraction of the cœnosarc, and the difference between this process and that shown by ordinary pieces appears to be due entirely to the fact that at the time when the tentacle primoidia are laid down, the cœnosarc has become free from the outer wall, or perisarc.

From this result it seems to me to follow with great probability that in ordinary pieces the closure of a cut end is also due to a process of contraction of the cœnosarc, but ordinarily the wall of the cœnosarc is so closely stuck to the inner surface of the perisarc that it is not free to pull away as a whole, and there is a consequent drag that holds back the contracting wall, and a consequent modification of the method of closure of the opening. This conclusion also fits in well with some facts observed at the time of closure of the pieces. Certain of the cells that appear to be more closely stuck to the wall are often left behind, or are retarded in their progress towards the center of the newly forming membrane. Thus the peculiar method of closure of *Tubularia* finds its explanation in the unusually close connection between the perisarc and cœnosarc. I have tried to show elsewhere[1] that this same connection may also be responsible for the characteristic "incomplete structures" of *Tubularia*, whose chief peculiarity is that their organs are full sized so far as they are formed.

Transpositional or Compensatory Regeneration of the Large Chelæ in some Crustacea.

Przibram[2] discovered in 1901 in the decapod *Alpheus* that it is possible to cause the small claw (chela) of one side to become

[1] "Some Factors in the Regeneration of Tubularia," *Roux's Archiv*, XIV., 1903.
[2] *Roux's Archiv*, XI., 1901.

the large claw by the simple operation of removing the large claw of the other side. At the next moult the small claw becomes the big one, and the newly regenerated claw becomes the small one. Zeleny[1] found in 1902 that a similar throwing over of the large operculum of the annelid, *Hydroides*, can be brought about by the same sort of operation. Wilson[2] in 1903 made some important additions to Przibram's work, using an American species of *Alpheus*. He suggested that the small claw is merely an arrested stage of development of the big claw, and that when the big claw is removed the check is at the same time taken away that holds back the development of the small claw. At the next moult the small claw becomes the large one, and the new claw the small one.

As yet no one has detected the nature of the correlation that causes the transposition, and this must obviously be the next step in advance. Wilson has suggested that the throwing over is connected with the nervous system, but the experiments on which he bases this suggestion appear to me to be capable also of another interpretation.

During the past summer I undertook some experiments which I hoped would give results bearing on this question, but the outcome has been almost entirely negative. Nevertheless, I shall venture to describe these experiments briefly, because if carried out on more suitable forms they will very probably throw some light on this exceedingly important subject.

Several years ago I found that by cutting the nerve of the leg of the hermit-crab, proximal to the breaking joint, the leg can then be cut off at any level beyond the breaking joint without the remaining part being thrown off at the base. By removing portions of the large leg at different levels, after first cutting the nerve at the base, I hoped to be able to discover whether the amount removed had any effect on the transposition of the large claw to the other side. It was also possible that the simple cutting of the nerve might have some effect, as Wilson's experiment seems to show. The result might also, as Wilson appears to believe, depend in part upon the degree to which the new nerve

[1] *Roux's Archiv*, XIII., 1902.

[2] Biological Bulletin, IV., 1903.

regenerated before the next moult. In practice, however, it is not possible to cut the nerve without cutting also the blood-vessels, and the injury to the latter may be as important as, or even more so, than that to the nerve.

The experiments were carried out with the hermit-crab and with the fiddler-crab, but were unsuccessful in both cases for different reasons. First, the transposition does not occur under any circumstances in the hermit-crab, as this and other experiments showed; and second, in the fiddler-crabs the muscles, etc., beyond the breaking joint degenerate after the operation. This caused the death of most of the crabs, and those that remained alive had only the outer shell of the leg beyond the breaking joint, and even this fell off in several cases. Since, however, the operation can be carried out in the hermit-crab without the outer part of the leg degenerating, it may be possible, in other forms that have the power of transpositional regeneration (in *Alpheus*, for example), to carry out this experiment successfully.

In both the hermit- and the fiddler-crab I also tried the effect of removing three of the walking legs on the same side of the body as the big claw, leaving the big claw uninjured, in order to see if the absence of the other legs might possibly affect the transposition. This did not succeed, because in the hermit-crabs, as I have said, the big claw does not throw over, and in the fiddlers the experiment had to be brought to an end before any of the crabs had moulted.

All of the individuals of the hermit-crab that I have examined were right-handed, and the shells in which they live have also right-handed spirals. It has been suggested to me that this is an adaptation, in so far that the right-handed hermit crab is placed to better advantage in a right-handed shell. Consequently, if this were true (and I am by no means certain that it is so), it would be disadvantageous for the hermit-crab to have the power of transposition after the loss of the big claw, and in consequence this power has not been acquired, or else, if it existed in the ancestors of the hermit-crabs, it has been lost. That there is really no basis for an argument of this kind is shown by the state of affairs in other decapods; in the lobster, for example. In the American lobster I have seen several cases in which the

big claw had been lost and a new one of the same kind was regenerating on the same side. Przibram has also described cases of this sort. This result is all the more interesting since in the lobster the big claw is present in some individuals on one side, and in other individuals on the other. It cannot be claimed in the lobster that one kind of claw represents an undeveloped stage of the other. In the regeneration of the claws, as especially well seen in the lobster, the particular type of claw is present, although not always fully developed, at an early stage, as Przibram has described, and as I have also found. No doubt the advocates of the view that all beneficial processes have been acquired because of the benefit conferred, will find in these cases of transposition of the big claw from one side to the other evidence of the acquirement of a useful process through natural selection, but I do not think that there is any connection of this sort in these cases.[1]

I have intimated above that the injury to the blood-vessels that run to the leg may be closely connected with the changes that take place in the leg, and account for the absence of transposition in those experiments of Wilson's in which the nerve of the small claw was cut (and presumably also the blood-vessel). My work on the fiddler-crab convinced me that cutting the blood-vessels, which seems nearly always to take place when the nerve is cut, brings about important changes in the condition of the leg. If my suggestion prove correct, namely, that the lack of transposition in Wilson's experiment is due to injury to the blood-vessel rather than to cutting the nerve, then it is possible that the whole phenomenon of transposition may be connected with the condition of the blood supply to the leg. After removal of the large claw more blood may be thrown into the vessel going to the small claw, and this may be the cause of the change that takes place.

[1] In this respect I am in entire agreement with Wilson.

NOTES ON REGULATION IN STYLARIA LACUSTRIS.

E. H. HARPER.

This paper is not concerned chiefly with regeneration, but more especially with some processes of regulation accompanying regeneration which were observed in the living animals. *Stylaria lacustris* L. is a well-known fresh-water oligochæte of the family Naididæ, whose members have the power of reproducing by self-division. The worm is six to eight mm. in length, and is easily recognized by its long prostomium or proboscis. The body is divided into three regions: (1) An anterior specialized region of five segments, containing the pharynx, which is marked by a yellow pigment distinct in appearance from the dark brown chloragogue layer of the rest of the alimentary tract. The first segment bears the long prostomium and eyes. The mouth is ventral and the pharynx is slightly eversible. The anterior region is further distinguished by the absence of the capilliform dorsal setæ, ventral setæ also being absent from the first segment. (2) The middle region, with an indefinite number of segments, containing the œsophagus and the crop, a dilatation between the seventh and eighth segments, and the stomach-intestine. The middle region ends in a budding zone of incompletely developed segments and embryonic tissue. (3) The specialized anal segment, somewhat longer than the rest and tapering in form, devoid of setæ, and with the gut lined with cilia.

Self-division takes place at a region posterior to the middle of the worm, from the seventeenth to the twenty-fifth segments, very commonly at the end of the twenty-first. The first indication of division is the appearance of a band of transparent tissue divided in the middle by a slight constriction. Embryonic tissue accumulates before and behind the septum between the segments forming an ectodermal thickening. These regions lengthen and soon become segmented, that in front of the septum forming the anal segment of the anterior zoöid, and the part behind developing into a pharyngeal region of five segments for the posterior zoöid. The zoöids remain united until completely

developed, a continuous band of fæcal matter being visible through the length of the worm. During rapid division another zone of fission makes its appearance, one segment in front of the one previously formed, making a chain of three zoöids.

This method of self-division is often compared with the more primitive type called fragmentation in which the parts separate before regenerating the ends. The physiological regeneration that takes place in self-division is the same in its results as regeneration from a cut surface. Asexual multiplication continues through the warmer months. In October and November the sexual organs attain maturity. Budding then ceases, and the power of regeneration is also diminished.

Methods.

Whole mounts of these worms have been made by the following method. As stated above, the results of this paper have been obtained from the study of the living forms, but when it has been desired to preserve specimens to show results of regeneration, difficulty was encountered in killing the animals in an extended condition, since they invariably become coiled up on application of the fixing fluid. A remedy for this was found by getting the animal extended in the angle between the beveled edge of a slide and a glass plate. A very little hot sublimate-acetic applied to the animal in this position will be drawn under the slide with considerable force and prevent the animal from moving. If as little liquid as possible be used, the animals may be killed in a perfectly extended condition. The animal should be placed on the glass plate in a small drop of water. If the slide is then moved up to the drop, the worm will become extended next to the glass, and held there with sufficient force to prevent its coiling when the killing fluid is applied.

I. Regeneration in the Asexual Forms.

Experiments. 1. A résumé of some general features of regeneration in *Stylaria*.

These forms including *S. lacustris* have been studied extensively, both in respect to their method of self-division and their regeneration. Section within the middle region is followed by

regeneration of the pharyngeal region of five segments from an anterior cut surface. Similarly, the anal segment is regenerated from a posterior cut surface, but within a shorter time. In regeneration a bud or knob of transparent embryonic tissue is first proliferated, which increases in length, develops a prostomium, and becomes segmented within a few days. Section within the anterior specialized region is followed by the restoration of the number of segments which were removed, from one to five.

Thus it is seen that the pharyngeal region of five segments is the unit of regeneration after section within the trunk region, and the process is identical with the normal or physiological regeneration that occurs in self-division. On the other hand, the segments are the units of regeneration when the mutilation is within the pharyngeal region.

There is a close analogy between these results and the observations made upon the earthworm, in which four or five segments are regenerated after more than five are cut off.

The prostomium has great power of regeneration. Frequent cases are met with of forms with regenerating prostomium. Child ('00) figured a specimen found in nature with a forked prostomium which was doubtless a product of regeneration. In Fig. 9 is shown a somewhat similar case of regeneration of the prostomium following the severing of the organ at the base; in this case the organ is doubled. In later stages the lateral bud was absorbed.

2. Regulation of the intestine behind a cut surface to form the œsophagus and crop.

It was stated at the outset that this paper deals chiefly with certain processes of regulation accompanying regeneration proper. As an excellent example of the general regulative changes accompanying regeneration in this worm a case of regulation may be mentioned which occurs normally in the process of self-division or physiological regeneration as well as in cases of regeneration after injury. The œsophagus and crop occur in the sixth to eighth segments, *i. e.*, not in the region which is formed from new tissue by proliferation. Hence the œsophagus and crop must be formed by a process of regulative transformation of the intestine behind the new proliferating tissue. This involves a

narrowing of the intestine to form the œsophagus and a dilatation to form the crop. The histological features are not considered here. The same process occurs in regeneration from a cut through the intestine.

3. Inhibition of the zone of fission under the influence of a regenerating region anterior to the fission plane.

A striking instance of regulation is seen in the effect produced upon a zone of fission by section anterior to it. Under certain conditions the formation of a regenerating region will inhibit the process of self-division and cause the disappearance of the zone of fission. This involves the rearrangement of tissues and a process of redifferentiation in a region at a distance from the cut surface. If the animal is severed in front of and near to a zone of fission which is in an early stage of development before segmentation has taken place, the constriction and the accumulated embryonic tissue disappear and the zone is completely redifferentiated (Fig. 1, *a–c*).

The nearer the regenerating region is to the zone of fission the greater is the tendency for the latter to disappear, but the influence may be exerted at a considerable distance. The following table gives the results in 48 cases:

Number of Segments Between the Cut Surface and the Zone of Fission.	Number of Experiments.	Disappearance of Zone of Fission in.
1–7	28	60 per cent.
8–15	20	15 per cent.

In all the cases included in the above table the zone of fission was in an early stage of development, but not all were at exactly the same stage. If only specimens were taken for experiment in which the zone was just beginning to form, no doubt a much larger percentage of cases of disappearance would be obtained both when the regenerating region was near and when it was far away.

The writer was informed by Professor Frank Smith of an observation upon a nearly related form, *Pristina* sp., to the effect that the zone of fission may disappear in animals kept in the laboratory, as a result of insufficient food or other unfavorable conditions. It is realized that a control experiment would be desirable, to show what percentage of cases of disappearance of

the fission zone might be traceable to other causes than the influence of the regenerating region. The results here given are, however, relied upon in the belief that the early disappearance of the zone of fission (in all cases in about two or three days) sufficiently indicated that it was due to the influence of the regenerating region. There is also the evidence of the cases to be mentioned later in which the zone of fission did not disappear subsequent to a cut behind the zone of fission.

An effect is produced the converse of what has just been described when an animal is severed in front of and near to a well-advanced zone of fission. In such a case its development exerts a retarding influence on the regenerating region. A small piece in front of such a zone of fission is soon separated, after undergoing little or no regeneration.

4. Effects of section behind a recently formed zone of fission.

Section behind a recently established zone of fission does not ordinarily seem to exert an inhibitive influence. In only one such case observed was the zone redifferentiated. No change is produced, unless it be a retardation of the development of the zone of fission. Two considerations seem to have weight in explaining the difference between this and the case in which the cut is anterior to the fission plane. First, the regeneration of the anal segment is a process requiring the withdrawal of less material from the old parts than the regeneration of an anterior end, and hence is calculated to interfere less with the development of the zone of physiological regeneration. Second, the establishment of a regenerating region behind the zone of fission does not materially change the conditions already existing in that region, since in this case a regenerating region posterior to the fission plane is substituted for the previously existing proliferating region in front of the anal segment.

Galloway ('99) has noted in respect to *Dero vaga* that cutting behind the fission zone tends to disorganize its growth and the posterior piece soon drops off.

As a parallel to the results of the preceding experiments upon *Stylaria* it may be mentioned that Dr. Child has informed me that he has found a similar disappearance of the fission plane in the Rhabdocœle *Stenostoma*, under the influence of a regenerating

region. There is probably a close similarity in the physiological processes connected with fission in the two groups.

5. Disorganization in the region behind a cut surface.

(*a*) There are a number of phenomena taking place internally during regeneration which can be plainly seen, owing to the transparency of these animals. When a portion of the head segment in front of the eyes is severed, in almost all cases the pigmented portions of the eyes are found later in a fragmented condition and the black pigment cells scattered through the pharyngeal region (Figs. 2, 5, 11). This does not seem to be a direct mechanical effect, since it occurs when the eyes are apparently not directly injured by the mutilation. This observation, which may seem of slight importance, is mentioned as showing how organs behind the regenerating region may be affected, apparently disorganized, in this case, and later restored. The eyes are reformed subsequently and the scattered pigment cells disappear. In Figs. 5 and 11 the eye appears in two parts. It became normal later. The interpretation, if such it may be called, which would seem to be indicated by the facts, is that the region just behind the cut surface tends to return to an embryonic or disorganized condition as the result of change in physiological conditions.

(*b*) Another observation of a like nature has been made in the case of the regeneration of the whole pharyngeal region. During the regeneration numerous brown pigmented cells, similar to the chloragogue cells of the stomach-intestine, are seen floating in the cœlomic fluid. These are different from the normal unpigmented cœlomic corpuscles. As was pointed out above, the region behind the cut surface is a seat of regulative activity, the œsophagus and crop being produced in that region by regulation of the intestine. The free pigment cells may be a product of the disorganization of the intestine immediately behind the cut surface, as in the case of the pigment cells of the eye.

As stated above, these two observations seem to indicate that the organs just behind the cut surface tend to return to an embryonic condition similar to that of the new proliferated material. This breakdown of tissues may also be comparable to the loss of cell boundaries and return to a syncytial condition in the well-

known experiments of Loeb with segmenting *Fundulus* eggs, in which the cause ascribed was lack of oxygen.

6. Healing of a cut surface without regeneration.

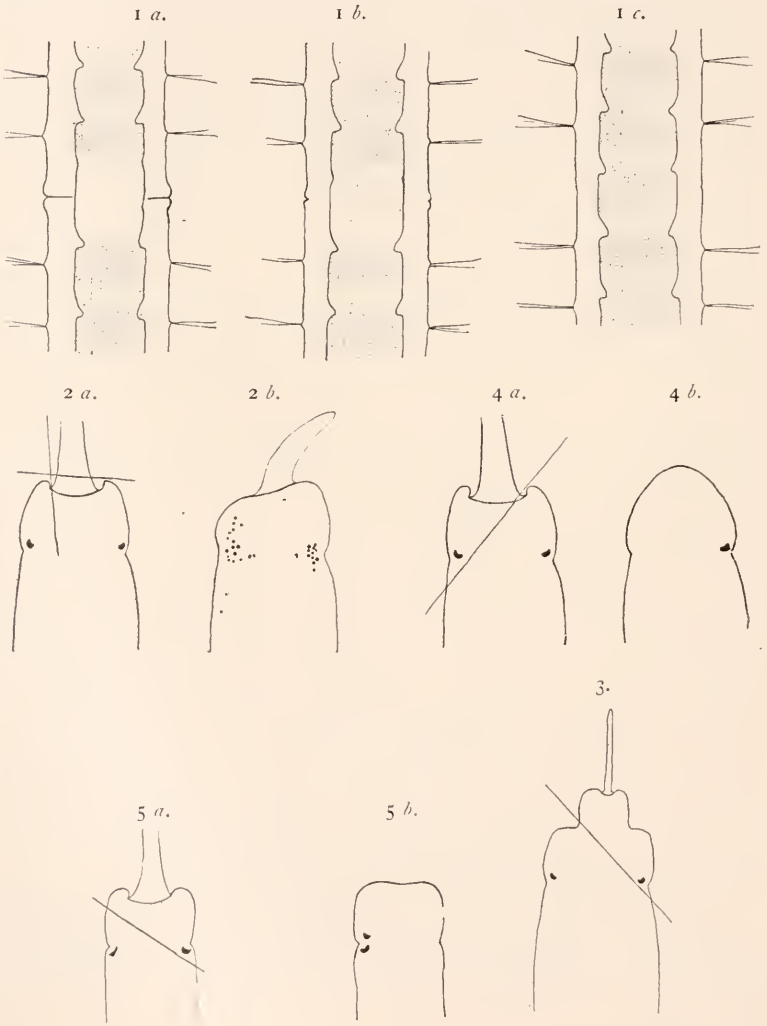
The regenerative power is so strong in the asexual naïds that it would naturally be expected that regeneration would take place under all circumstances in pieces above a certain limit of size. Certain instances of failure to regenerate have been met with, however, which indicate that the power to regenerate is dependent upon certain internal conditions. If an oblique cut be made in front of the pharynx, removing the prostomium and one side of the head segment, including perhaps one eye, the cut surface may frequently heal over and failure to regenerate the prostomium may result (Figs. 4 and 5). A number of such cases have been obtained. In some instances the animals were kept for more than three weeks without showing any tendency to regenerate the prostomium. Since regeneration ordinarily requires only a few days, the effects seemed in these cases to be permanent.

Internal conditions favorable to proliferation, such as the exposure of cut surfaces of intestine and blood-vessels, are present in nearly all possible experiments with *Stylaria*. But after section of the prostomium and one side of the head without touching the pharynx, the tendency to proliferate is apparently at a minimum. The ectoderm may, therefore, close over the wound and cut off the outlet for proliferating material. Failure to regenerate seemed to follow only after an oblique cut, but in one instance, after a transverse cut in front of the eyes, a somewhat similar result was obtained. In this animal an outgrowth appeared from the cut surface which was of less diameter than the region behind, appearing like a new head segment superimposed upon the old. Its appearance suggested that the outlet for the proliferating material had been narrowed by reduction of the cut surface. It is conceivable that still further reduction of the cut surface, or even complete closing of the wound, might occur in case the proliferating tendency was slight (Fig. 3).

The above cases of failure to regenerate were only occasional instances, showing that the balance between the tendencies to proliferate and to heal over without regeneration is rather deli-

cately adjusted. Cases occurred in which the prostomium was regenerated, but the side of the head or lobe was not normally restored, at least within the period of observation (cf. Fig. 13, of a sexual individual).

The general conclusion is that certain parts which may be mutilated without exposing cut surfaces of internal organs which are of importance as internal factors in proliferation are less likely to be regenerated, since, when the proliferating tendency is slight, the ectoderm closes over the wound and checks proliferation.



Description of Figures.

Fig. 1. (*a*, *b*, *c*.) Redifferentiation of a zone of fission. In (*a*), a zone is formed and there is a constriction in the body-wall. The intestine in this region is transparent, devoid of pigment. In (*b*) and (*c*) the zone and constriction have disappeared and the intestine becomes pigmented. The accumulation of transparent embryonic tissue before and behind the septum in (*a*), mentioned in the text, is not indicated in the figure. The ectodermal thickening is indicated at the constriction. In this and all the figures the dorsal capilliform setæ are not represented full length.

Fig. 2. Fragmentation of the eyes after mutilation. The prostomium was severed and a short longitudinal cut made in the first segment. The direction of the cut indicated by line. The condition in (*b*) was observed three days after the mutilation. The slightly curved outgrowth of the bud of the prostomium is an unusual occurrence.

Fig. 3. Regeneration from first segment, resembling a repetition of the head segment, of less diameter. The line indicates direction of the cut. The eye is regenerated. The condition here represented was fifteen days after the operation.

Fig. 4. Failure of regeneration after an oblique cut through first segment. In (*b*) the condition twenty-three days after the operation is shown. The eye is not regenerated and there is no trace of a prostomium.

Fig. 5. Failure of regeneration in first segment. The eye in (*b*) is fragmented into two portions. Later it became normal.

7. Absence of regeneration in short posterior pieces.

With the purpose of finding whether heteromorphosis would take place, as in the earthworm, in the case of very short posterior pieces a number of experiments were tried with as short posterior pieces as would survive the operation. No tails appeared on the anterior ends. A number of pieces which survived the operation for as long a time as three weeks, did not regenerate the anterior ends. During the first week these pieces elongated within the budding region in front of the anal segment. The immature segments became further differentiated, their setæ increasing in size, but no new segments appeared. The pieces then appeared to be composed of segments nearly equal in size, and there was no zone of embryonic undifferentiated tissue remaining. One such piece was obtained by a cut one segment behind the pharynx of a posterior zoöid. It contained at first a region composed chiefly of indistinct segments. At the end of a week these segments had increased in length and their setæ had grown until twenty segments could be easily counted. Its size was greater than that of a minimal piece capable of regeneration taken from the middle of the body. It showed no trace of anterior regeneration for three weeks, but was still active in its movements. Un-

fortunately, it was not possible to keep it under observation longer. A number of similar pieces have been kept with the same results (Figs. 6 and 7). The elongation of such pieces did not seem to be merely mechanical, since it was accompanied by some differentiation such as the growth of the segments. Possibly the proliferating tendency at the cut surface was checked by the continued growth of the budding zone. The latter, containing an accumulation of embryonic tissue, was perhaps able to check regeneration by absorbing the available materials for growth. Whether such pieces would in time regenerate needs, of course, to be determined before the possibility of heteromorphosis would be settled.

8. Regeneration of the posterior end.

It was thought possible that heteromorphosis might occur in regeneration from short anterior pieces. In no case did the specialized anterior region of five segments survive long enough to begin regeneration. An anterior piece of eight segments survived for six days without showing any posterior regeneration. This was the shortest piece which was able to survive more than a short time after the operation. Pieces with only two more of the trunk segments present on the average regenerated freely, however, as the following table will show:

Length.	Number of Experiments.	Number Able to Survive for Several Days.	Number Able to Regenerate.
8–9 segments.	9	2	0
10–11 "	10	9	7
12–13 "	10	9	9
14–29 "	37	35	33

There is a great average increase in the power to regenerate correlated with the presence of only two additional segments of the trunk. The specialized head region, as would be expected, has the least power of regeneration. Portions of the trunk of the same length as the first class given in the above table are able to regenerate freely.

No cases of true heteromorphosis were obtained. The anterior region of five segments which might perhaps be expected to develop a head posteriorly, contains no nephridia (they are found back of the sixth segment), and as we have seen, does not survive the operation long enough to regenerate. Results obtained in

Planaria, with its diffuse excretory system, could not be expected to occur in a form like *Stylaria*, with a head region so specialized as to be unable to exist independently even for a short time.

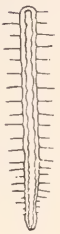
A case of duplication of parts was seen in the production of a double-headed individual, one of whose heads was larger and in line with the axis of the trunk. The other was lateral, lying close to it, and smaller. The smaller one was in process of absorption at the last stage at which it was observed (Fig. 8).

A doubling of the prostomium was noticed in one case as mentioned above. The two organs lay parallel and were fused together except at the tip of the shorter (Fig. 9). In later stages the lateral bud was absorbed.

9. Regulation of growths oblique to the main axis.

It is a common observation that proliferation tends to take place along an axis normal to the cut surface. After an oblique cut the new growth continues oblique until it has reached its full length or nearly so. The straightening of such oblique growths is evidently not a function of growth alone, but of growth influenced by certain tensions, which may be exerted by the part behind the new growth. Rievel ('96) has pointed out the influence exerted by the peristaltic motions of the intestine upon the regeneration of the head region. The knob of new tissue is at first solid and is later penetrated by the slower ingrowth of the lumen of the intestine into the region. The waves of peristaltic motion constantly passing along the intestine favor its ingrowth into the new region. Finally the mouth is formed by the bulging out of the body-wall, caused by peristalsis. It is broken through from within, there being no stomodæal invagination of the ectoderm in regeneration.

It may be suggested that peristaltic movements of the intestine have an influence in straightening oblique growths. This is probable since the straightening occurs not during the proliferating period, but coincidently with the ingrowth of the pharynx. Straightening occurs, moreover, while the tissue still appears embryonic before the complete differentiation of muscular and nervous systems (Figs. 10 and 13).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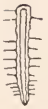
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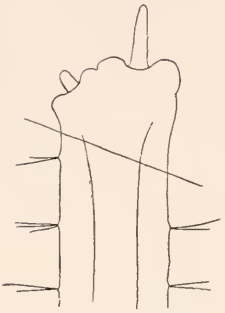
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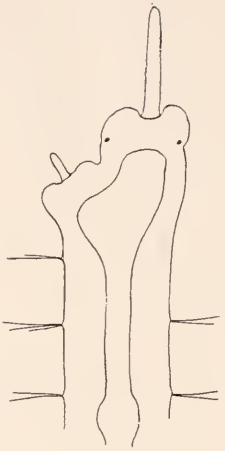
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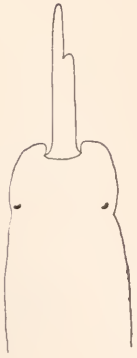
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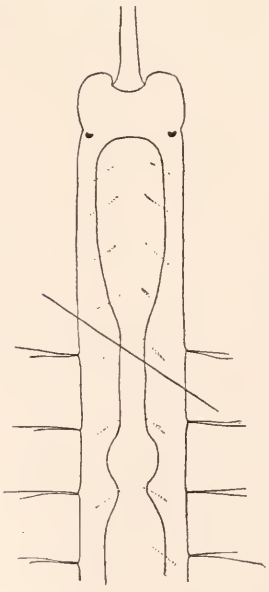
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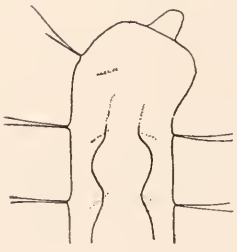
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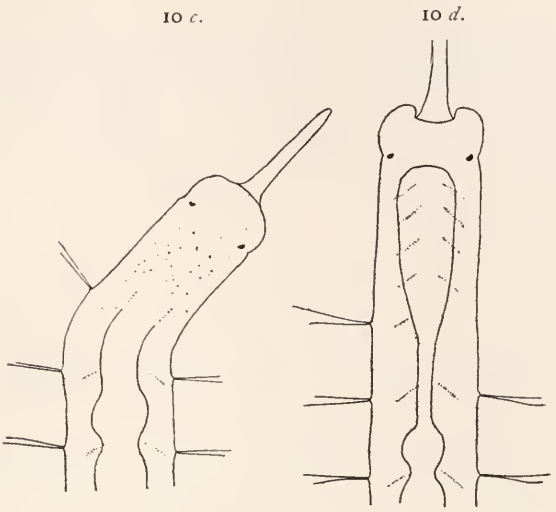


DESCRIPTION OF FIGURES.

FIGS. 6 and 7. Failure of anterior regeneration in case of very short posterior pieces. Fig. 6 represents such a piece twenty-three days after it was severed, Fig. 7 a piece eighteen days after it was severed. It measured 1.25 mm. Further observation was prevented.

FIG. 8. Regeneration of a double-headed individual. The line in (*a*) indicates direction of cut. The smaller head was apparently destined to be absorbed at last observation. The specimen was lost before regeneration was complete. Stages (*a*) and (*b*) were six and ten days after the cut was made.

FIG. 9. Regeneration of abnormal prostomium, a doubling of the organ. Later the lateral bud was absorbed.

FIG. 10. Regeneration occurring in a direction normal to the cut surface. (*b*) represents the condition three days after the operation, (*d*) the complete regeneration. In (*d*) the right dorsal bundle of setæ is not restored. In (*c*) scattered pigment cells resembling the chloragogue cells are present in the new tissue.

II. REGENERATION OF THE SEXUAL FORMS.

The sexual organs of *Stylaria* come to maturity during the months of October and November. In the height of the sexual stage the animals cease to multiply asexually. In the earlier stages budding animals are frequently seen. In the fully developed sexual animal the formation of new segments in the anal proliferating region also ceases. As a result the animals attain larger size, since the segments in the budding region become fully differentiated. The clitellum is conspicuous, covering the fifth and sixth segments, in which the testes and ovaries lie. This part becomes more opaque and loses its setæ.

The results obtained in regard to the regeneration of the sexual forms will be treated under two heads : (1) The regeneration of the pharyngeal region involving the results following the severing of 1–5 anterior segments ; (2) regeneration within the trunk region.

1. In the regeneration of the pharyngeal region three sorts of cases are met with :

(*a*) Complete regeneration may occur in the less mature sexual forms.

(*b*) There may be an incomplete regeneration, resulting in the production of an outgrowth which is devoid of ventral setæ and seems to be the equivalent of the first segment. The regenerated part is distinguishable by its relative lack of pigment and by the fact that it does not attain the original diameter (Fig. 11). It is comparable to Fig. 3, where a growth took place in an asexual form from an apparently restricted outlet, and the same explanation may be offered here, viz., that owing to the slowness of proliferation the partial closure of the cut surface by the body-wall restricted the outlet. The prostomium does not reach full length and the lateral lobes of the head are not developed. Often the eyes are not formed in such cases.

(*c*) Often complete failure to regenerate occurs. The end heals over and a mouth is formed (Figs. 12 and 14). In twenty-one cases of mature sexual animals in which from one to four segments were removed there were six such cases of failure to regenerate and the rest regenerated an amount apparently equivalent to one segment.

2. After the removal of five or more segments results followed which likewise may be divided into three classes.

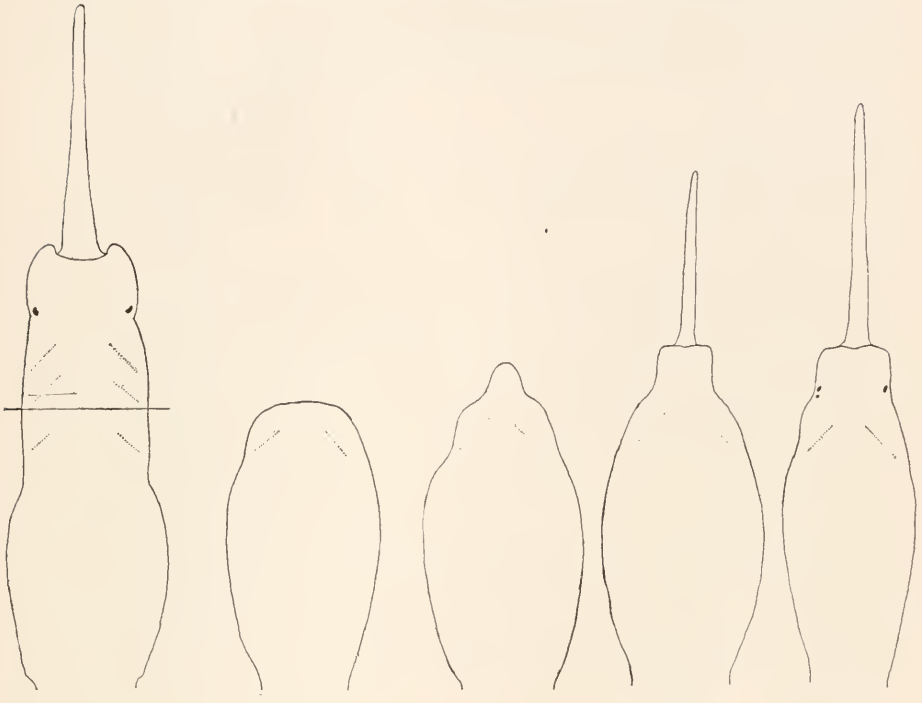
(*a*) Normal regeneration in the less mature sexual specimens.

(*b*) A pharyngeal region of five very short segments with a diminutive prostomium may be formed. Four pairs of ventral setæ appear showing that the full number of segments is represented, but the region is remarkable for its dwarfed appearance (Fig. 15).

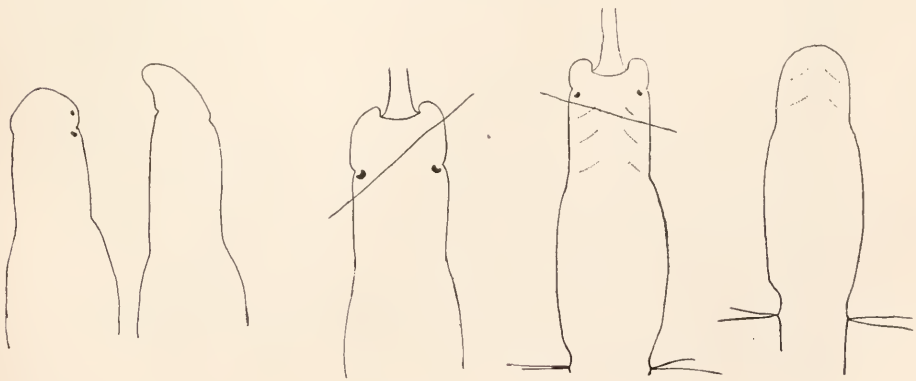
(*c*) There may also be failure to regenerate. In twenty-nine cases in which from six to thirty segments were removed there were six cases of failure to regenerate anteriorly and five cases of the dwarfed regeneration.

The regeneration of the anal segment is equally slow in the advanced sexual forms and the part regenerated is not of full size (Fig. 16).

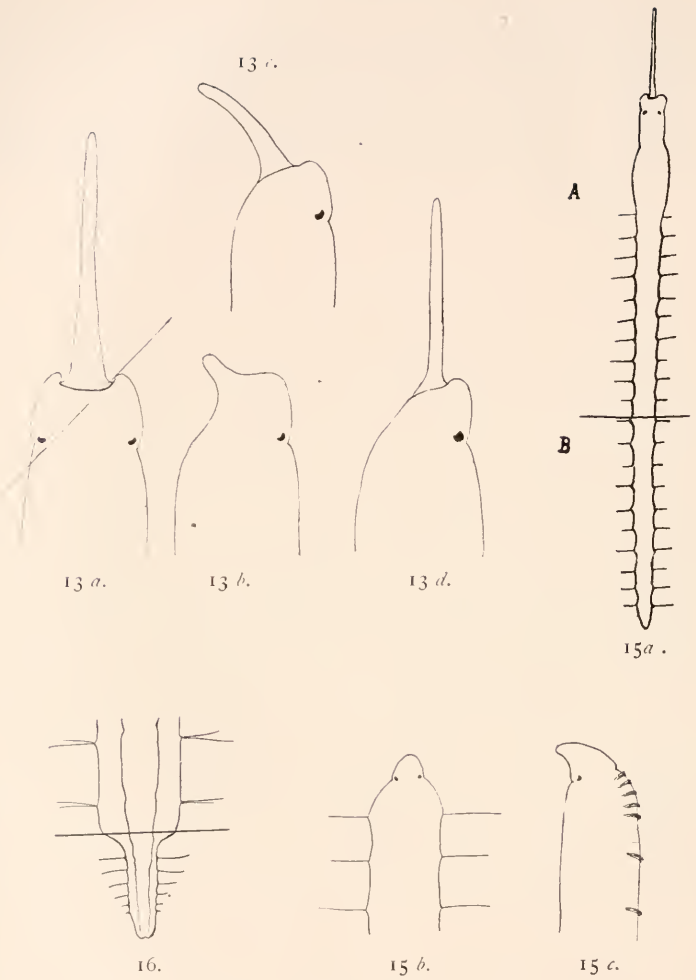
3. In a single instance the clitellum was regenerated, but the specimen was unfortunately not kept under observation long



11 *a*. 11 *b*. 11 *c*. 11 *d*. 11 *e*.



12 *c*. 12 *b*. 12 *a*. 14 *a*. 14 *b*.



DESCRIPTION OF FIGURES.

FIG. 11. Regeneration of a sexual individual after removal of first three segments. The clitellum is indicated by the widening of the body. It takes in the fifth segment, which is a part of the pharyngeal region. The setæ are absent from the clitellum. One pair of ventral bundles were left in front of the clitellum. In stage (*e*) the head segment is regenerated. As no more pairs of setæ bundles were produced, the regenerated part is regarded as the equivalent of the head segment alone. The stages (*b–e*) followed 3, 8, 12 and 19 days after the operation, respectively.

FIG. 12. Failure of regeneration after severing part of first segment in a sexual individual. In (*b*) a side view is given. The condition shown in (*b*) and (*c*) followed seventeen days after the cut.

FIG. 13. Oblique growth of bud of prostomium in (*b*), and slightly incomplete regeneration. Stages (*b*, *c*, *d*) were 4, 8 and 18 days after the operation, respectively. The eye was not regenerated. The other eye increased in size.

FIG. 14. Failure of regeneration in a sexual individual after removal of two segments. This condition existed nineteen days after cut was made.

FIG. 15. Dwarfed anterior regeneration from a sexual individual after removal of fifteen segments. The prostomium is not regenerated. A pharyngeal region of five short segments is produced, as shown by the number of setæ bundles. In (*c*) a side view is given. The level at which the cut was made is shown in (*a*).

FIG. 16. Regeneration from posterior end of a sexual individual. The part produced is of less diameter.

enough to determine whether the regeneration of the sexual organs was complete. The cut was made just behind the clitellum.

CONCLUSIONS.

1. The formation of a regenerating region will under certain conditions inhibit the process of asexual multiplication and cause the disappearance of the zone of fission. This effect may be produced by a cut anterior to the zone of fission, less often by a cut posterior to it, and occurs only when the zone is embryonic. The zone is also more likely to disappear if the cut is near to it. The band of transparent embryonic tissue redifferentiates and the energy of growth is transferred to the regenerating region.

2. There is evidence of disorganization and a return to embryonic conditions in organs just behind a cut surface. This effect does not appear to be a direct mechanical result, *i. e.*, due to crushing. Fragmentation of the pigmented portion of the eye is one case adduced.

3. Internal conditions favorable to proliferation, such as the exposure of cut surfaces of intestine and blood-vessels, are present in nearly all possible experiments. But if a corner of the head segment be removed, including the prostomium, without injuring the pharynx, the ectoderm may close over the surface and regeneration may fail to take place.

4. Short posterior pieces often fail to regenerate anteriorly, but no cases of heteromorphosis, such as individuals with tails on the anterior end, have been obtained. Short posterior pieces which failed to regenerate within the time of observation may elongate and show some differentiation in the budding region.

5. The middle portion of the body has the greatest power of regeneration, the specialized pharyngeal region has the least.

6. Growth takes place at right angles to a cut surface, and if the cut is oblique the bud will grow out at an angle to the axis of the body. Straightening is affected after the penetration of the lumen of the pharynx into the region, probably under the influence of the tension produced by the peristaltic motions of the intestine.

7. Sexual individuals lose the power of regeneration to a large extent just as they do their power of budding. In an advanced sexual stage regeneration may fail to occur or be incomplete.

I wish to express my thanks to Dr. C. M. Child for suggesting to me the subject of this paper and for further suggestions relative to some of the results described.

Hull Zoölogical Laboratory,
University of Chicago, 1903.

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VARIABILITY IN THE NUMBER OF TEETH ON THE CLAWS OF ADULT SPIDERS, SHOWING THEIR UNRELIABILITY FOR SYSTEMATIC DESCRIPTION.[1]

CARL HARTMANN.

Since the number of teeth on the claws of spiders is often used as a specific character, the need of testing its constancy suggested itself. It has been pointed out by W. Wagner [2] that the number of ungual teeth varies with each moult. In the present study of the variations in the adult this fact was well taken into consideration, great care being exercised in choosing fully mature individuals that had undergone the last moult. To my knowledge no one has ever tested the constancy of the number in fully mature individuals.

The study was made on the claws of the right legs of 70 females and 40 males and comprises, therefore, observations on nearly 1,320 claws of 440 legs. Representatives of a number of different families were chosen as follows: Dictynidæ (*Dictyna volupis*, Keys., West Chester, Pa.), Theridiidæ (*Theridium tepidariorum*, Koch, Philadelphia), Pholcidæ (*Spermaphora* sp.? Hentz, Austin, Texas), Epeiridæ (*Epeira marmorea* Clerck; *E. benjamini*, Walck.; *Acrosoma reduvianum*, (Walck), West Chester, Pa.), Lycosidæ (*Lycosa nidicola*, Emerton, Austin, Tex.).

The counting of the claws was easy except in the case of *Dictyna volupis* and of *Spermophora;* but even here, if any mistakes in the counting are recorded, they are extremely few, for I never left a claw until convinced that my count was correct; or in a few cases where this seemed impossible the individual was entirely discarded. To count the teeth the three claws of each leg, if they were large, were snipped off with a needle (keeping the foot on a slide in a drop of alcohol) and pressed flat with a cover-glass. If the claws were too small, the whole tarsus was

[1] Contributions from the Zoölogy Department of the University of Texas, No. 55.

[2] W. Wagner, "La Mue des Aragnées," *Ann. Sc. Nat.*, 1888, p. 363.

Table I. Females.

Species	No. of Leg.	No. of the Individual. 1	2	3	4	5	6	7	8	9	10
Dictyna volupis.	1	13,9 5	13,12 5	13,11 5	12,11 5	12,11 5	12,10 5	14,12 5	14,12 5	12,12 5	13,11 5
	2	12,10 5	———	12,12 5	12,10 5	11,— 5	12,12 4	12,12 5	12,11 5	13,13 5	11,12 5
	3	9,10 4	10,9 4	–,9 4	9,9 4	9,9 3	8,9 4	9,10 3	10,10 4	10,10 3	9,9 4
	4	9,10 4	9,10 4	10,10 4	9,10 4	9,10 3	9,9 4	11,10 4	9,10 4	10,11 4	10,10 4
Theridium tepidariorum.	1	6,6 2	6,5 2	5,8 2	6,5 2	6,5 2	6,6 2	4,5 4,5 2 2	6,5 –	6,5 2	6,6 2
	2	6,6 2	6,5 2	6,5 3,2	6,5 2	6,6 2	6,5 2	5,5 –,6 2 2	7,5 2	6,6 2	6,6 2
	3	5,6 2	5,6 2	5,5 2	5,6 2	5,6 2	5,6 2	–,5 –,6 2 2	5,6 2	5,6 2	6,6 1
	4	4,2 2	3,2 1	4,2 1	4,2 2	4,2 2	4,1 1	3,2 3,2 1 1	4,2 1	3,1 2	4,2 1
Sphyrnophora.	1	11,10 1	10,10 1	11,11 1	10,10 1	10,10 1	10,10 1	11,10 1	11,10 1	9,9 1	11,10 1
	2	10,10 1	10,10 1	11,10 1	———	12,10 1	10,10 1	11,10 1	11,11 1	9,9 1	10,10 1
	3	9,10 2	10,10 1	11,11 1	10,10 1	11,11 1	9,9 1	10,9 1	10,10 1	9,9 1	9,12 1
	4	7,9 0	7,9 1	–,9 2	9,10 1	7,9 1	8,10 1	8,10 1	8,9 1	7,8 1	10,10 1
Epeira marmorea.	1	7,8 2	8,8 2	7,8 2	8,9 2	9,7 2	7,8 3	7,8 3	8,8 2	8,8 3	9,9 3
	2	7,8 2	6,7 2	7,8 2	9,9 2	8,8 2	8,7 2	7,8 3	7,8 2	8,8 2	8,8 3
	3	6,6 2	–,7 2	———	6,7 2	7,6 2	6,5 2	6,6 2	6,7 1	6,6 2	8,7 2
	4	———	5,6 2	6,5 1	6,8 2	6,6 2	5,6 2	6,5 2	6,5 2	6,6 2	6,7 2
Lycosa nidicola.	1	4,4 0	4,4 0	4,4 0	4,4 0	4,4 0	5,4 0	5,4 0	4,5 0	4,4 0	4,4 0
	2	7,6 0	7,6 0	5,4 0	5,4 0	5,4 0	6,5 1	5,4 0	5,6 0	5,5 0	4,5 0
	3	———	7,6 0	6,6 0	7,6 0	6,5 0	7,7 0	7,6 0	7,7 0	7,7 0	6,6 0
	4	6,5 0	9,9 0	6,7 0	6,7 0	5,7 0	8,8 0	7,7 0	8,8 0	7,7 0	7,7 0
Epeira benjamini.	1	5,8 2	5,9 2	8,– 3	8,8 2	9,8 3	8,8 2	10,8 3	8,9 2	7,8 2	6,8 2
	2	5,9 2	6,9 2	5,6 3	7,9 2	7,9 3	8,8 2	8,9 2	7,8 2	6,8 2	7,8 2
	3	3,6 2	8,7 2	7,6 2	7,7 2	8,7 2	7,7 2	6,11 2	7,6 2	7,7 2	6,6 2
	4	6,5 2	7,6 2	7,6 2	4,5 2	6,5 2	5,5 1	6,5 2	6,5 2	6,5 2	6,5 2

TABLE I. FEMALES.—*Continued.*

Species	No. of Leg.	No. of Individuals.									
		1	2	3	4	5	6	7	8	9	10
Acrosoma reductinum.	1	7,6 2	7,6 2	7,7 2	7,6 2	7,7 2	7,6 2	8,7 2	7,6 2	7,7 2	7,7 2
	2	——	7,6 2	7,6 2	7,6 2	7,– 2	6,6 2	6,7 2	7,7 2	7,7 2	6,7 2
	3	5,6 2	5,5 2	6,5 2	6,5 2	6,6 2	5,5 2	6,6 2	5,5 2	6,6 2	5,5 2
	4	3,2 1	3,4 2	3,4 2	4,4 2	——	4,5 2	5,4 2	4,2 1	4,4 2	4,3 1

covered, placed under the compound microscope and pressure applied until all the teeth came into full view.

The results of the counting of the teeth are recorded in the accompanying tables, Table I. containing the figures for the females and Table II. for the males. The three claws were always distinguished from one another and the number of teeth on each recorded after the formula $\frac{a, p}{i}$, where a represents the number of teeth on the anterior, p on the posterior and i on the inferior claw.

The reduction in the number of teeth seems to take place at the proximal end of the claw because, firstly, the distal teeth usually maintain the size and form characteristic of the species, and secondly because the proximal tooth (or teeth) in some cases becomes so small as to merit the name tubercle in place of tooth. This latter fact forced me to establish a criterion to determine what to count as a tooth and I decided to call the structure a tooth if it had attained a length at least half as great as its width at the base.. In *Dictyna volupis* the two distal teeth are small and are closely approximated to the claw for nearly their entire length. One of these was counted in some four or five cases where it was unusually large and stood out from the claw for at least half its length.

In order to reduce the tables to percentages so as to get at a simple set of figures for comparison I have adopted what may be called the "percentage of constancy" method, which may be illustrated as follows:

In Table I. the anterior claw of the first leg of *Lycosa nidicola*

Table II. Males.

Species	No. of Leg.	No of the Individual									
		1	2	3	4	5	6	7	8	9	10
Dictyna volupis	1	12,11 5	12,11 5	12,10 5	12,11 5	12,9 5	11,11 5	11,11 5	10,10 4	11,11 7	11,10 6
	2	12,12 6	12,11 5	13,12 5	11,11 5	12,11 5	12,11 5	11,– 5	11,11 5	12,11 6	11,10 6
	3	11,11 4	11,10 4	9,9 4	9,10 4	9,9 4	9,9 4	10,10 5	9,9 4	–,10 4	10,10 5
	4	11,11 5	10,10 4	9,9 4	10,10 4	10,9 4	10,10 4	11,10 4	9,10 4	10,11 5	10,10 5
Theridium lepidariorum	1	4,4 1	5,5 1	5,4 2	5,5 2	6,4 2	–,4 2	5,– 2	5,5 2	5,5 2	5,5 2
	2	4,4 1	5,4 2	5,4 2	5,4 2	5,4 2	5,4 2	5,4 1	5,5 2	5,5 2	5,5 2
	3	–,4 1	4,5 2	5,3 2	4,5 2	3,5 2	4,3 2	4,5 2	4,3 2	4,4 2	5,5 2
	4	2,1 1	3,1 1	3,1 1	3,1 1	4,1 1	3,– 1	4,1 1	4,1 1	3,1 1	4,1 1
Spermophora	1	12,11 1	12,– 1	12,11 1	12,10 1	11,10 1	10,9 1	10,10 1	11,10 1	11,10 1	12,11 1
	2	12,10 1	11,10 1	12,11 1	11,10 1	12,11 1	10,9 1	11,10 1	10,10 1	11,10 1	11,10 1
	3	10,11 1	11,11 1	11,10 1	–,10 1	11,11 1	9,9 1	10,10 1	9,9 1	10,9 1	10,10 1
	4	9,10 1	8,10 1	8,10 1	8,10 1	9,10 1	8,9 1	8,9 1	8,9 1	———	9,10 1
Epeira marmorea	1	7,9 2	10,11 2	9,8 2	8,9 2	8,9 2					
	2	8,8 2	7,9 2	8,8 2	8,8 2	———					
	3	6,6 2	6,6 2	7,8 2	6,6 2	6,6 1					
	4	6,6 2	7,– 2	–,5 1	6,5 1	6,6 1					
Lycosa nidicola	1	6,6 0	6,6 0	6,6 0	6,7 0	6,6 0					
	2	7,7 0	8,8 0	7,7 0	7,8 0	7,8 0					
	3	8,9	9,10 1	8,8 0	8,8 0	9,9 0					
	4	8,9 0 0	9,9 0	———	9,9 0	10,10 0					

has 4 teeth 8 times out of 10, the posterior claw has 4 teeth 9 times out of 10. So that the claws have percentages of constancy of 80 and 90 respectively. On the second leg of the same species the anterior claw has 5 teeth more often than any other number of teeth or in other words it has a maximum constancy of

5 in 10 = 50 per cent.; the posterior claw has a maximum constancy of 40 per cent. having 4 teeth 4 times in 10. This method was pursued throughout. Where only five specimens of a species were examined the result was calculated to ten. On the basis of these data the following conclusions were drawn. Where percentages are cited they are percentages of constancy and refer only to the superior claws.

1. Claws having larger numbers of teeth show more variation in numbers than claws having a smaller number and this holds for both sexes. The percentages of constancy for the superior claws of male and female are as follows : *Spermophora* 52 per cent., *Dictyna volupis* 54 per cent., *Epeira marmorea* 59.5 per cent., *Lycosa nidicola* 62 per cent., *Theridium tepidariorum* 68 per cent. A glance at the tables will convince one that this order will practically hold for the relative total number of teeth.

The same result is more strikingly shown by the inferior teeth. In *Dictyna volupis*, which has normally 4–5 teeth on the inferior claw, there are 16 variations in 80 cases of both males and females ; while *Spermophora* and *Lycosa nidicola*, which have normally only 1 and 0 tooth respectively have only 3 variations in 80 cases in the former case and 1 in 60 in the latter.

Moreover, the superior claws, having many teeth, show many times as much variation as the few-toothed inferior claw.

2. The teeth on the claws of the first leg show least variation in number, those on the third most. The percentages of constancy are : First leg, 61.5 per cent.; second leg, 60.0 per cent.; third leg, 57.0 per cent.; fourth leg, 58 per cent.

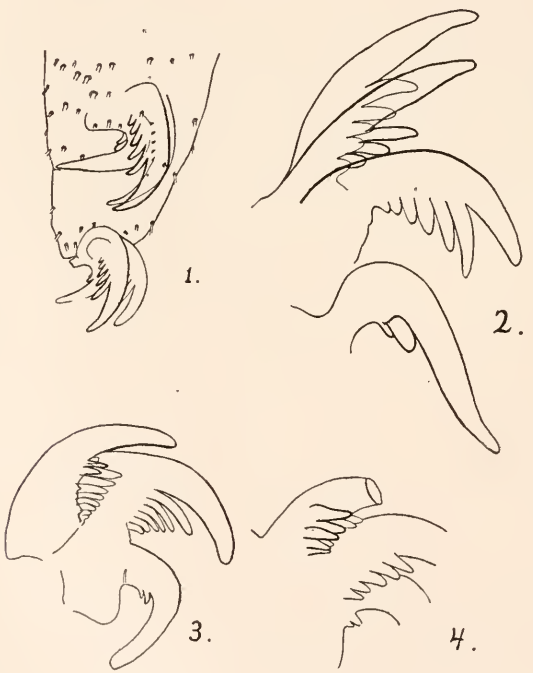
3. The number of teeth on the claws of the female varies slightly more than in the male, the percentages of constancy being 58.0 and 59.75 respectively. The inferior claw gives the opposite result, however, for here in the females there are 17, in the males 19 deviations from the normal in 160 cases. A glance at the tables will disclose a sexual dimorphism in the total number of teeth which may be greater in the male or in the female or the same in both, according to the species. Thus by actual count 5 females of *Epeira marmorea* have 318, 5 males 335 ungual teeth on the right side ; but 5 females of *Lycosa nidicola* have 318 teeth as against 228 for 5 males ; while 10 females and 10 males of *Dictyna volupis* have each 1,030 teeth exactly.

4. The anterior and the posterior claws vary in nearly equal measure throughout, the average constancy percentage for the former being 58.0, for the latter 59.75.

5. In only one case were two individuals found that had the same number of teeth on the corresponding claws of all the four legs on the right side (Table II., *Theridium tepidariorum*, individuals 2 and 4).

In view of these facts one would be safe in saying that the number of teeth on the tarsal claws of spiders is too variable to be used as a specific character; it should at least not be used in a diagnosis until its absolute constancy for a given species has first been demonstrated.

In addition to this common variation in the number of teeth on the claws one often meets with additional claws or with double



rows of teeth on the same claw (mutations). Fig. 1 represents the first foot of the seventh specimen of *Theridium tepidariorum* in Table I. Two complete sets of claws, similar to the ones

figured for the first leg, were found on each leg of the animal, both on the right and on the left side. These claws were all of the normal type but possessed relatively fewer teeth than those on the claws of other specimens of the same species.

Fig. 2 represents four claws on the second leg of the second female of *Theridium tepidariorum*, the claw bearing three teeth being an additional inferior one.

Figs. 3 and 4 show on the posterior claws double rows of teeth, the additional row lying close to the normal and more regular one. The claws in Fig. 3 are from the third leg of specimen seven of *Epeira benjamini* and those in Fig. 4 from the fourth leg of specimen four of *E. marmorea*, both in Table I.

In conclusion I wish to thank Dr. T. H. Montgomery for the many specimens which he placed at my disposal and for his kind suggestions in my work.

University of Texas,
Austin, Texas, December 24, 1903.

A BIOLOGICAL EXAMINATION OF DISTILLED WATER.

E. P. LYON.

The accuracy of results obtained in many of the present lines of physiological research depends so entirely on the purity of the chemicals used, including water, that I may be pardoned for publishing an account of work which otherwise would have little significance, the toxicity of metal-distilled water having been repeatedly shown. The results obtained are, however, applicable in a practical way at Woods Hole, where it is difficult to get good distilled water.

Two years ago I arranged an automatic still in which the water was boiled in a block tin vessel but the dry steam received and condensed in glass. It was thought that if the water did not touch metal after condensation, contamination by ions would be avoided. The distillate proved, however, to be decidedly toxic. I could not believe that dry steam carried with it an appreciable quantity of metal and, therefore, decided to find out where the trouble lay.

In these experiments, sea-water was condensed to a known fraction of its volume and then measured portions brought back to the original volume by adding the distilled waters to be tested. Tap-water was also used for comparison; sometimes, without treatment; sometimes, sterilized without boiling; in some experiments a portion was boiled away and the remaining portion used.

To the artificial sea-waters prepared as above equal amounts of fertilized *Arbacia* eggs were added and the development observed. The percentage of plutei developed in the different solutions as compared with natural sea-water was usually taken as a measure of purity. In other cases, the percentage of blastulæ was the standard; or the percentage of segmentation in a given time; or the length of time that any larvæ remained alive. The records of some typical experiments follow:

Experiment 6.—250 c.c. sea-water were concentrated to 75 c.c. To each 15 c.c. of this solution 35 c.c. of the waters named

in column 2 were added. To the dishes were added equal amounts of *Arbacia* eggs in 16–32-cell stage.

No	Description of Water.	20 Hours.	28 Hours.	54 Hours.
1	Double distilled in glass.	Early gastrulæ. Behind control.	Behind control.	Plutei.
2	Tap.	Blastulæ.	Many going to pieces.	All dead.
3	Tap boiled down one half.	Slightly behind No. 1. But much better than No. 2.	Almost as good as No. 1.	Plutei as well developed as No. 1; not so numerous.
4	Control in sea water.			Advanced plutei.

Experiment 11. — Two liters of sea-water were evaporated to 250 c.c. To 10 c.c. portions of the resulting solution were added respectively 70 c.c. of waters described below. Equal quantities of *Arbacia* eggs were added. Three days later the percentages of plutei were ascertained by first killing the cultures and then making counts in a watch glass marked off into squares.

	Per cent. Plutei.
1. Control, 80 c.c. sea-water.	90
2. Artificial sea-water made from tap-water.	0
3. " " " " " tap-water heated to 60° in closed flask.	0
4. " " " " " distilled water from Metcalf's, Boston.	16[1]
5. " " " " " water boiled in copper without H_2SO_4 but condensed in glass.	3 2[1]
6. Artificial sea-water made from water boiled in copper with H_2SO_4 but condensed in glass.	78[2]
7. Artificial sea-water made from water distilled wholly in glass; first part thrown away.	66
8. Artificial sea-water made from water distilled in patent automatic metal still.	5[3]
9. Artificial sea-water made from water distilled wholly in glass, with $K_2Cr_2O_7$ and H_2SO_4.	87
10. Artificial sea-water made from water double distilled in glass, first distillates rejected.	90
11. Artificial sea-water made from "Pureoxia" distilled water.	3[4]

Experiment 8. — Two liters of sea-water were boiled down to 400 c.c. Ten c.c. of this concentrated sea-water were added to 40 c.c. of each of the waters listed in column 2. To every dish was added an equal amount of fertilized *Arbacia* eggs on July 10, 1903.

[1] Nearly all small.

[2] Some small; others as good as control.

[3] All very small and imperfect.

[4] All very small and imperfect. Only experiment with this brand.

EXPERIMENT 8.

No.	Description of Water.	Condition of Cultures on Dates Named.					
		July 11.	July 12.	July 16.	July 20.	July 23.	July 28.
1	Tap.	No larvæ.					
2	1st 50 c.c. distilled in glass from 1,000 c.c. tap.	Many gastrulæ, but not so good as No. 6.	Many plutei; not so good as No. 3.	Some alive; not so good as No. 4.	All dead.		
3	2d 50 c.c. ditto.	Ditto.	Not so good as control. 40 per cent. plutei.	Many alive.	Some alive.	All dead.	
4	3d 50 c.c. ditto.	Better than No. 2.	As good as control.		Many alive.	Many alive.	All dead
5	Distilled in glass from tap water, which had been boiled in open vessel.	Fine lot of gastrulæ.	As good as control.	Many alive and lively.	All dead.[1]		
6	1st 50 c.c. distilled from 1,000 c.c. tap + 5 grms. potassium bichromate and 10 c.c. H_2SO_4.	Ditto.	Better than control.	Much better than No. 3 or No. 9.	Many alive; not so good as No. 8.	Many alive.	All dead.
7	2d 50 c.c. ditto.	Ditto.	Ditto.	Ditto.	Ditto.	Ditto.	All dead.
8	Double distilled in glass, first distillate in each case thrown away.	Ditto.	As good as control or better.		Fine lot alive.	Fine lot alive.	All dead.
9	Distilled water from Metcalf, Boston.	Ditto.	About like No. 2.	Many alive.	All dead.		
10	Same as No. 5 + 1 c.c. $\frac{m}{10}$ NH_4OH	No development.					
11	Control, in 50 c.c. sea-water.	Fine lot of gastrulæ.	50 per cent. are good plutei.	Some alive.	All dead.[1]		

[1] Not usual in water prepared in this way. Should have lived longer.

From the above and other experiments I can make the statements enumerated below, which apply, of course, to *Arbacia* only but point to the necessity of caution in using distilled water on other organisms. It is true also that the results are most applicable to Woods Hole conditions and to the tap-water used as a basis of the distilled water there. It is probable that the toxicity is due to ammonia although this was not proven. It is certain from experiments made that *Arbacia* larvæ are very sensitive to that substance.

1. Tap-water is decidedly, although variably, toxic. The toxicity is not lost by sterilization but is greatly reduced by boiling the water for a long time, say until one third has been boiled away. The residue in such cases is less toxic than some distilled waters, particularly commercial brands and that from automatic stills.

2. Water from ordinary automatic stills, whether metal or entirely glass, is toxic.

3. The commercial distilled waters used by me were toxic, often in high degree.

4. In distilling water in the ordinary way from glass, the first one tenth distilled over is decidedly toxic, the second tenth less so, the third tenth still less so. The fourth tenth is of good quality.

5. The best distilled water used was produced by double distilling in glass, the first fourth distilled over in each distillation being rejected.

6. Nearly as good water was produced by single distillation from tap-water to which H_2SO_4 and $K_2Cr_2O_7$ had been added.

7. If a little H_2SO_4 is added to the tap-water to start with, an excellent quality of distilled water may be produced from an automatic still consisting of a copper vessel and glass condenser, the arrangement being such that none of the condensed water touches the metal. This water is practically free from ions or toxic volatile substances. It is much better than water double distilled in glass in the ordinary way, unless in the later case a large proportion of the product be thrown away. Such an automatic still is recommended for use at Woods Hole.

8. It was noted in a number of cases that *Arbacia* lived longer

in an artificial sea-water prepared from a good quality of distilled water than in natural sea-water. It is probable that the volatile toxic substance (ammonia?) exists in sufficient quantity in sea-water to have an appreciable effect.

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BIOLOGICAL BULLETIN

ON THE HABITS AND REACTIONS OF SAGARTIA DAVISI.

HARRY BEAL TORREY.

Sagartia davisi[1] is the Pacific representative of the *S. luciæ* of the Atlantic coast of the United States. The two species appear to differ only in the absence, in *S. davisi*, of the narrow yellow stripes on the column which are so characteristic of the eastern form. Both species commonly reproduce by fission, and it is probable that the following descriptions of some of the other habits of *S. davisi* may be applicable to *S. luciæ* also.

S. davisi was first discovered clustered on the valves of a species of the bivalve *Chione*, common in the harbor of San Pedro, Cal.[2] The clam dwells near or on the surface of the sand. As it plows its way along, the uppermost regions of the shell are about the hinge and the siphons. To these regions, invariably, the polyps cling. The deeper the clam goes the more do their small, thin-walled bodies lengthen, supported by the surrounding sand, to a degree I have never seen in an erect and unsupported form. *S. davisi* is not, however, a burrower, nor is the association with *Chione* a case of commensalism, as might be concluded at first sight. It fastens readily to any object which can give it a foothold and keep it out of the sand. On the sand flats at San Pedro

[1] Description. Column of largest polyps about 1 cm. in diameter; spread of tentacles about 2 cm. Foot disk very extensible; body wall everywhere quite thin and semitransparent; a distinct capitulum above a well-defined collar as in *Metridium;* oral disk almost circular, mouth small, oval, lips with about twelve lobes, not prominent; one or two, occasionally three siphonoglyphs. Tentacles tapering, slender, pointed, variable in number, most often 45–50 in perfect individuals. Color of column dark brown, tentacles and disk green. Many individuals with light longitudinal stripe of variable width on column (zone of regeneration after fission). Mesenteries unusually variable in number and arrangement. Reproduction sexual and non-sexual; latter the cause of irregularities in number and arrangement of tentacles and mesenteries.

[2] It has since been found in San Diego Bay, Cal.

it was uncovered about half each day by the falling of the tide, during which periods its tentacles were completely retracted, contrary to the custom of such typical sand dwellers as *Harenactis attenuata*,[1] and another species of *Sagartia* from San Pedro as yet undescribed. It may live permanently submerged, however, and thrives in aquaria.

Though ordinarily attached to a solid substratum, it is occasionally found free on the sand. Its powers of locomotion are considerable. By means of multicellular amœboid processes of the foot disk, readily seen with a hand lens at the edge of the disk, it is capable of creeping more than an inch in an hour. The polyps often leave the clam to which they are attached when placed in an aquarium, especially when they are on the lower valve. They may occasionally creep along the surface of the water, hanging from the surface film.

The inverted position is not long retained, however, for *S. davisi* has a marked tendency to assume as erect a posture as its situation will permit. An example of this tendency is provided in the case just mentioned, of the relatively greater haste of the polyps in leaving the lower than the upper valve of a clam lying on the aquarium floor. Moreover, the axes of polyps clinging to the vertical sides of the aquarium are either perpendicular to the sides or bent upward. They never bend downward if the polyps are submerged.

The orientation of *S. davisi* is, then, partly a result of geotropic stimulation. The same may be said of the locomotion of the species. There is a definite tendency of the polyps on the walls of the aquaria to collect near the surface, although the aquaria may be sealed jars completely filled with water, or furnished with green water plants evenly distributed (precautions against the possible influence of oxygen at the surface). The polyps on the floor of the aquarium, if it be horizontal, move about but little, and when they do, sporadically and without certainty of direction. When by chance, however, they reach the angle made by the floor and a side of the aquarium and begin an ascent, there is never a retrograde movement, seldom a halt, until they draw near the surface. This locomotor geotropism is especially inter-

[1] Torrey, 1901.

esting from the fact that the major axis of the animal is not parallel with the direction of locomotion, a peculiarity which distinguishes it from the reactions of the majority of animals to directive stimuli. The major axis is the axis of geotropic orientation, but it can only be the axis of locomotion in swimming forms [1] and those which lack a foot disk and creep on the column (*e. g.*, *Peachia*).

It is possible to reconcile these different cases if we think of the foot disk merely as a differentiated portion of the body wall. *Edwardsia* has no well defined foot, though its aboral end is rounded and adhesive. The hydroid, *Corymorpha*, again, has no foot such as is possessed by *Hydra*, its aboral end coming to a point; yet the sides of this tapering extremity are adherent, and through their amœboid cells the hydroid, orienting negatively to gravity, tends also to move vertically upward. It adheres in this case by a portion of what may truly be called its lateral wall; in consequence of which the axes of locomotion and geotropic orientation coincide. *S. davisi* is an extreme case in the other direction. Having a large and well defined foot, it can hardly be said to cling obviously by a portion of the lateral (*i. e.*, column) wall. At the same time, when on a vertical surface, its axes of locomotion and orientation are as nearly parallel as the differentiation of foot and column will allow. This, however, is not equivalent to saying that the direction of locomotion in response to a directive stimulus is determined by the orientation of the major axis of the polyp, for the elements of the foot may be directly affected by gravity.

Loeb ('91, p. 70) has said that *Cerianthus* and *Actinia equina* went from smooth glass to a mussel shell or piece of ulva more readily than in the reverse direction in his experiments. This indicates a certain "contact irritability," which seems to be possessed also by *S. davisi*, as the latter moves about more freely on smooth glass than on rough surfaces. The reaction to the contact stimulus, however, is not so strong as the orienting re-

[1] Besides the pelagic species which Andres describes among the *Minyadidæ*, all non-adherent, there is an interesting polyp abundant in the harbor of Honolulu which, I am told by my friend Mr. Loye H. Miller, leads both a sedentary and a free existence. It appears to have no pedal float, sustaining itself by means of rhythmic movements of the tentacles which send it along foot foremost at a fair rate of speed.

action in response to the stimulus of gravity, so that as a resultant of the opposing responses, the polyp leaves the shell for the glass as stated above.

Light does not appear to stimulate *S. davisi* in any way. The polyps neither bend nor move toward the light when it comes from but one side of the aquarium, in all degrees up to the intensity of bright daylight. Neither do flashes of sunlight falling upon polyps in a darkened aquarium produce any muscular responses. *S. davisi* differs in this respect from *Cerianthus membranaceus* and *Edwardsia lucifuga*, according to Nagel ('94, p. 545).

The responses of anemones to mechanical and chemical stimuli have been investigated already by Pollock ('82), Loeb ('91 and '95), Nagel ('92, '94*a*, '94*b*) and Parker ('96). With most of the conclusions of these investigators my own observations accord. I must differ with some, adding also a few facts which to my knowledge, have not been published heretofore.

Two quite distinct reactions usually follow the stimulation of a tentacle of *S. davisi* by means of a slight touch with a needle or glass rod. The first is a bend at and toward the point of stimulation, whether the latter be near tip or base, on right side or left, above or below, and appears to be due to the response of the muscles involved to a direct stimulus. The second is a contraction of the whole tentacle, with a simultaneous bending of the tentacle toward the mouth. Evidently all the longitudinal muscles of the tentacle not previously active are indirectly excited to produce this reaction, those on the inner (upper) side between base and point stimulated contracting more strongly than the outer (lower) muscles. This unequal contraction is probably to be explained by the greater strength of the inner muscles, which play the greater part in the chief work of the tentacles — carrying food to the mouth. The hydroid *Corymorpha* shows this inequality still more strikingly; the first reaction of *S. davisi* is entirely wanting and the outer muscles are in use only when the tentacles are slowly returning to their expanded condition after a contraction. But I have been unable to demonstrate histologically any difference in size between outer and inner muscles, in either animal.

The result of the second reaction is varied. Often the tentacle merely waves stiffly inward. At other times it may arch so that its point is directed toward the mouth. On the whole, however, its movements are less definitely adaptive than those which Parker describes for *Metridium*.

The second reaction does not always follow the first. The general contraction does not appear to be induced by contact alone. If the tentacle be touched lightly and for but an instant, only the first reaction occurs. If, however, the stimulating object rest against the tentacle sufficiently long to allow the latter to adhere to it, the second reaction immediately follows. Whether this results from the adhesion itself, or the duration of the stimulus, or a tension in the muscles due to the resistance of the stimulating object, I am unable fully to decide. Such small objects are capable of producing this reaction that the third possibility seems to be excluded. Whichever of the other two be the efficient stimulus, it produces a strong contraction of the muscles directly affected. This strong contraction probably serves as a direct stimulus for contiguous muscles, the contraction of these for others, and so on, until all are involved. In no case did the evidence enforce the assumption of the presence of nerves, in the tentacular responses.

So far the movements of but a single tentacle have been considered, without relation to the others. And it should be said here that tentacles cut from the polyp behave in all essential respects as they do under normal circumstances. Often the stimulus applied to one tentacle is sufficient, unless care be used, to induce contractions in several. It may be that only a few tentacles on each side of the one touched will react; with a stronger stimulus the entire set of tentacles may contract with vigor. There is no more evidence, however, that this correlation of parts is attained by the aid of nervous tissue than there was in the case of the single tentacles. Communication from one tentacle to the next is largely through the oral disk. The proper degree of contraction of a tentacle induces a contraction in neighboring muscles in the oral disk, and possibly in contiguous tentacles directly. The vigor of the stimulus, if it be local, appears to determine the extent of the

response, which spreads by the direct effect of the tension of one muscle on those near it.

The usual response of a tentacle stimulated indirectly in the manner just described is a waving or arching toward the mouth, with or without vigorous shortening of the whole tentacle. Occasionally the response is quite opposite to this, the stimulation of one tentacle producing an outward waving of neighboring tentacles. The anomaly is only apparent, not real, for as a matter of fact the muscles of the neighboring tentacles are not involved at all in the latter case. The tentacles neither shorten nor bend. They move outward stiffly, owing to a local contraction of muscles in the oral disk or the capitulum.

If the stimulus applied to a tentacle be sufficiently strong, all of the tentacles may shorten simultaneously, may even be entirely withdrawn into the body by the contraction of mesenterial muscles and hidden by the contraction of the sphincter; the column may shorten also, and the foot disk may change its shape. All of these movements seem to be induced by the direct passage of the stimulus from muscle to muscle without the aid of nerve tissue.

The oral disk, between tentacles and mouth, is almost insensible to mechanical stimuli.

Stimuli applied to the column produce the inward movement of several or all tentacles, the outward movement of a few, or the contraction of column and foot disk, according to the strength of the stimulus. Stimulation of the foot disk, either at the edge or on the lower surface, produces local contraction of the foot and base of the column, and acontia are usually emitted near the point stimulated. The tentacles may contract also, but always as a whole, the same general reaction following stimulation at different points of the disk instead of a local reaction as in the cases of the foot, column and acontia. This inability of the tentacles to recognize the direction of the stimulus is also characteristic of the reaction of the tentacles of *Corymorpha* to stimulation of the column, and is due, I believe, to the opportunities for diffusion of the stimulation impulse owing to the distance of the reacting structures from the point at which the stimulus is applied.

The entire surface of *S. davisi*, with the possible exception of

a small zone between mouth and tentacles, responds to a mechanical stimulation, the greatest irritability being manifested by the tentacles, the tactile organs *par excellence*. The latter exhibit a very definite adaptive reaction. The preliminary bend of an irritated tentacle at and toward the point stimulated makes it possible for the polyp in a sense to pursue its prey actively if, indeed, to but a limited extent. The great advantage of this reaction over the simple inward movement of the tentacle indirectly stimulated is obvious. The latter is also adaptive, however, since it is the most likely movement to clutch food organisms in a polyp whose tentacles are habitually outstretched. Supporting this idea is the fact that the tentacles of hydroids react only in this way, whether stimulated directly or indirectly. It is the simpler, more primitive reaction.

A more efficient adaptive reaction, also indirectly induced, is the extrusion of acontia at the point stimulated, for purposes of defense. Though the reaction is always the same, the acontia always move in the most desirable direction, which is not always the case with tentacles.

The passive outward movement of tentacles due to the contraction of muscles in the oral disk or capitulum is not directed by the position of the stimulating object. It may be toward the latter, but only when the stimulus happens to be applied at a point external to the tentacles, that is, at some point which is less likely to be stimulated by a food organism than points on their inner surface. When a tentacle of an outer whorl is moved passively as a result of the stimulation of a tentacle of an inner whorl, the movement is away from, not toward the tentacle stimulated. The reaction in this direction is of no obvious importance to the polyp in this case, and seems to be of no more importance, in the sum of all cases, than a movement in any direction. It appears, therefore, to have no adaptive value whatever.

By means of its varying sensitiveness to different chemical substances and its ability to discriminate between mechanical and chemical stimuli, *S. davisi* is enabled to make certain choices in its quest for food. This capacity, which it possesses in common with other anemones, has been described as olfactory by Romanes

and Nagel, and as gustatory by Jourdain. Such expressions, however, are essentially psychological, and Loeb ('91) has justly insisted upon the substitution for them of some physiological expression, such as *chemical irritability*. This power of discrimination has been shown by Loeb to reside not only in the tentacles (Nagel, '92), but also in other regions. *Actinia equina* discriminated between crab's flesh and small rolls of paper as definitely after he had removed the tentacles by a transverse cut as before. Parker ('96) demonstrated later that *Metridium dianthus* reacts in different ways to mechanical and chemical stimuli.

Actinia equina, from Loeb's observations, is so definite in its choices that chemically inert paper pellets were never taken into the mouth. Parker found that *Metridium* would swallow pieces of white india rubber as well as flesh, though the former were sometimes disgorged before they had passed out of the œsophagus. Since he has shown that the cilia covering the lips of *Metridium* and beating outward in the absence of chemical stimuli, reverse their dominant beat in response to the stimulation of meat juices, their behavior when stimulated by apparently chemically inert india rubber leaves a doubt as to whether or not they can be reversed by purely mechanical means. There is, however, no doubt of such a reaction in *S. davisi*, as will be shown in the course of the following account of my experiments.

It may be well to begin with the effects of various chemicals. Cane sugar in solutions of various strengths produced no appreciable reactions in any part of the polyps on trial. Strong picric acid and 4 per cent. formalin caused the retraction of all the tentacles, indicating stimulation of body muscles. One half per cent. hydrochloric acid caused a general contraction of tentacles. From a knowledge of the behavior of *Corymorpha*, which, though unable to detect the presence of flesh until touched by it, yet reacts strongly to strong alcohol and acetic acid, I am led to suspect that these substances irritate the polyp in the same way that they irritate one's skin, through the tactile organs merely.

Crab's muscle, bits of limpet and annelid worm were used as

stimulators with uniform results. Small amphipods were devoured with avidity. The response differed according as the stimulus was applied locally or generally. For my first experiment I placed a small piece of worm on several of the outstretched tentacles of a polyp. The tentacles immediately adhered, bending at and toward the point stimulated, as though responding to a purely mechanical stimulus, and then contracted, dragging the morsel to the mouth. For some seconds the tentacles not in contact with it remained motionless. Then, one or two at a time, they waved slowly inward and grasped the flesh, almost every tentacle finally becoming thus engaged. This experiment was tried many times with similar results. Apparently the tentacles not mechanically stimulated were irritated by substances in solution diffusing out of the flesh, and the reaction was as definite as it would have been if induced by a mechanical stimulus. The movement was toward the stimulus.

The possibility of an indirect stimulation of these tentacles through the oral disk from the tentacles touching the flesh was eliminated by holding a piece of worm flesh immediately above the mouth of another polyp. In a few seconds some of the tentacles began to twitch slightly, and a little later all began to wave slowly inward, toward the flesh, finally grasping it. A similar result followed numerous trials.

Next, a bit of flesh was placed on the aquarium floor, near, but not in contact with the foot disk of another polyp. Would the tentacles bend in the direction of the flesh now, or toward the mouth? This experiment, repeated a number of times, did not give uniform results. In the majority of cases the tentacles waved toward the mouth, *away from* the flesh. In the rest they moved *toward* the flesh in the most definite and unmistakable manner. Not only that; the column, in several cases, bent toward the morsel which was seized by the tentacles nearest it and dragged toward the mouth. There is no doubt here that the movements were in the direction of the stimulating object, and are thus comparable to the well-known movements of the manubria of various mendusæ toward stimulated points on the subumbrella. The proboscis of *Corymorpha* reacts similarly, as will be shown in a forthcoming paper.

Pollock ('82) observed this fact but was unable to reconcile his varying results. The reason for his failure was, I believe, that he failed to distinguish between *general* and *local* stimulation. When the meat juices of the annelid used previously were discharged gently over a polyp from a pipette, I observed that the tentacles always waved inward, without regard for the direction from which the juice was coming. This general chemical stimulation produced the same response from the tentacles that a mechanical stimulation of the foot disk provoked. In both cases then, in which the tentacles waved inward and away from the flesh, the diffusion of soluble substances from the latter was probably so rapid that the tentacles were stimulated *on all sides* so nearly at the same time that no differential of stimulation beween opposite sides of the tentacles was established, the necessary condition of a directive reaction. But why the movement toward the mouth? Because it is the primitive clutching movement already spoken of as most likely to capture food organisms, in a polyp whose tentacles are habitually outstretched. It is the simplest adaptation of the prehensile mechanism, common to hydroids as well.

The responses of the tentacles to mechanical and chemical stimuli are essentially the same. The bend is toward the stimulus when the stimulation is local, toward the mouth when it is general, whether direct or indirect.

If we turn now to the phenomena of swallowing, we shall see that the cilia of both lips and œsophagus may respond to mechanical as well as chemical stimulation by waving more strongly inward than outward. I early observed that not only were pieces of flesh occasionally rejected, but bits of shell and gravel were sometimes taken in. With the idea in mind that the size and shape of the object might affect the reaction, several substances, presumably chemically inert, were given to various polyps, in pieces varying in these respects. Pieces of very thin paper, from 1 mm. to 3 mm. square, when placed npon the tentacles, were cast off in half an hour. A piece of cork, about one fourth as large as the polyp, was likewise rejected. A much smaller piece, capable of being easily ingested, was taken into the gullet and retained for thirty minutes. A piece of paraffine of similar

size was swallowed in three minutes, and a half-cube of heavy drawing paper, of about the same size, was also swallowed, though more slowly. Tiny bits of glass were frequently swallowed.

I can say definitely that these objects were not carried in by the beat of the cilia covering the siphonoglyphs and producing an insetting current, but by cilia covering the lips and œsophagus between the siphonoglyphs and producing a current which ordinarily sets outward. It would seem, then, that chemically inert substances, if small enough to be taken easily into the mouth and thus brought into direct contact with the ciliated cells lining the œsophagus, are ingested under some conditions. Other experiments show that one of these conditions, probably the most important, is the degree of hunger of the polyp. Starving polyps were always more ready than well fed individuals to swallow chemically inert substances. Some explanation of this fact may be derived from the further fact that hungry polyps are in general unusually sensitive to both chemical and mechanical stimuli. Increased sensitiveness means increased effectiveness of a given stimulus; this is equivalent to saying that the stimulus is more intense. *S. davisi*, then, responds only to certain intensities of the same stimulus, so far as the ciliated cells of the lips and œsophagus are concerned. Under mechanical stimulation of a given intensity, the cilia do not reverse their beat; an increase in the intensity or, if you will, effectiveness of the stimulation may produce this reversal. To chemical stimuli, or to mechanical which are above a certain degree of intensity (*i. e.*, when the stimuli polyp is starving), the response is usually positive; to a weakened mechanical stimulus there is less likelihood of any response.

The positive response to mechanical stimuli is undoubtedly advantageous to the polyp. It is apparent that substances with even a very small food value must be of some importance to a starving polyp although they would not be desirable as food for a well nourished animal. For the latter they would come into the category of useless substances, which the ciliary currents on œsophagus, lips and tentacles are admirably adapted to remove.

The disgorgement of non-nutritious bodies may now be briefly considered. All harmless non-nutritious bodies, and all food stuffs from which the nutrient juices have been taken during the process of digestion, are sooner or later cast out of the mouth. The cause of the ejection is to be found in the behavior, under varying stimulation, of the œsophageal cilia. The mesenterial filaments bordering the mesenteries, and the defensive filamentous acontia, are ciliated, but probably take no part in the process, for several reasons.[1]

First, the mesenterial filaments pursue excessively meandering courses along the edges of the mesenteries, and their cilia produce many currents which are antagonistic instead of proceeding in one general direction. I have not been able to determine whether the cilia beat more strongly away from or toward the mouth. In all parts of each filament, however, they appear to beat in the same direction ; and this beat is not reversed by contact with meat or meat juices. Second, the cilia on the acontia beat always more strongly toward their free ends, and they too do not reverse their beat in the presence of meat juices. Since the acontia are attached by one end only, have a marked tendency to coil, and occupy without regularity of arrangement any position in the cœlenteron, they can hardly be concerned with the phenomena of disgorgement. It may be noted in passing, however, that when they are thrust through mouth or cinclides, their cilia, in carrying toward their tips whatever foreign particles may come in contact with them, are performing what must be in the long run an advantageous service.

Finally, the œsophagus itself, ordinarily more than half the length of the column, reaches nearer to the foot disk when the polyp contracts as it does with food substances within it. The objects taken into the cœlenteron never get far away from the lower edge of the œsophagus. Under the influence of the mesenterial and acontial cilia, they may, if small enough, rotate aimlessly about during the period of digestion and absorption. In the absence of direct stimulation, the œsophageal cilia resume

[1] The following facts concerning the behavior of the cilia on mesenterial filaments and acontia were obtained from *Metridium*, but I feel confident that the same results would have followed an investigation of *S. davisi* had the supply of material permitted.

their dominant outward beat, and are able to carry away non-stimulating objects. At the end of the period of digestion and absorption, the ingested bodies have reached their minimum of stimulating power; and now, no longer able to reverse the dominant beat of the œsophageal cilia, they are carried out by the latter just as soon as they come into their sphere of influence. Why chemically inert bodies, once swallowed, should be disgorged, may be explained, I believe, by assuming inability on the part of the œsophageal cilia to continue reversing their dominant beat in the presence of a persistent or frequently applied mechanical stimulus which was originally weakly positive. This is in entire harmony with Parker's demonstration that after repeated applications of a weak chemical stimulus to the lips of *Metridium*, there comes a time when no positive reaction results.

Peristaltic movements of the œsophagus may assist the cilia, but I have no evidence that they take more than a very subordinate part in the phenomena of swallowing or disgorgement.

University of California,
January 11, 1904.

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VARIATION IN BEES.

FRANK E. LUTZ.

In the study of evolution, there is nothing more important than the investigation of variations, since the whole doctrine rests upon the premise that organisms do vary. There was a time when it was sufficient in such an investigation to take a series of specimens and from the general looks of things postulate theories. But the world has become more critical now and demands that when a statement is made concerning some phenomenon, exact data accompany the statement. Hence, the statistical study of variation which attempts to exactly measure the variations and correlations of different organs and to set them down in figures which "cannot lie." And here we cannot allow the other proverb which says that figures will prove anything, for figures truthfully handled can only prove the truth. But there is, on the other hand, great danger that, having collected a set of measurements, we make a show of accuracy that will lead us and others astray by reason of careless or insufficient analysis. Such work is most troublesome because of its seeming exactness and the difficulty of detecting errors.

Messrs. Casteel and Phillips, in the December (1903) number of this BULLETIN, have taken up a very interesting and vitally important problem. The comparative variability of the drone and worker bees hits, in a way, at the very root of the variation question. Accordingly, while we lament with the authors the smallness of their series, it seems well worth while to consider a few points about the paper.

In the first place, we have to disagree with the statement that if the variability is "due to chance," it is "not in accordance with any law," for it is well known, and has been for years, that nothing is more bound by law or more expressible in mathematical formulæ than "chance." However, we will heartily agree with them that the "true test of the relative variability" is the "descent in numbers of individuals" in the different classes as they are removed from the mean; but we wonder greatly

why they did not apply this simple test. It is called the standard deviation and must be known to everyone who has ever done any statistical work. The phrases just quoted are taken from the discussion of the counts of the hooks on the hind wings. Let us therefore examine them by means of this confessedly better measure.

We find that for the drones we have:

Lot.	No. of Specimens.	Standard Deviation.	Probable Error.
I.	50	2.1548	0.1453
II.	100	1.5435	0.0736
III.	100	1.7716	0.0845
IV.	100	1.6486	0.0786
V.	50	2.0988	0.1416
VI.	98	1.9377	0.0934

For the workers we have:

Lot.	No. of Specimens.	Standard Deviation.	Probable Error
I.	50	1.5223	0.1027
II.	350	1.5564	0.0397
III.	100	1.5523	0.0740

This gives an average standard deviation, or variability, for the drones of 1.8592 ± 0.1028; and for the workers of 1.5437 ± 0.0721. But we see that the difference between the averages for the two sexes is less than the difference between the two sets of drones from the same hive (I. and III.); and, considering the probable errors, neither is significant. If we omit the three small series because of their large probable errors, we see that the difference between the variabilities of the two sexes is even smaller and clearly not significant. It is also unfortunate that the work should have been passed by both the authors and still two of the nine averages be wrong. The average for lot II. of the workers is 20.99, not 21.08; and that for lot VI. of the drones is either 22.65 or 22.75, according as we do or do not include the individual with 12 hooks, but it is surely not 22.42. This was probably gotten by including this individual (although he was excluded by their argument above), and then using 100 as the total number, but for some strange reason there are only 99 of the 100 said to have been studied which are listed.

Passing over the grave error of lumping the different series of ratios (p. 27) because they seemed to be alike, when really their only claim to homogeneity is that they are of the same sex and all bees — Italians, hybrids, "peculiar strains," *et al.*, from central Ohio to eastern Pennsylvania being jumbled together — let us take up the first table (vein R) as we have done the last. We find that the standard deviation of lot I. of the workers is 1.5637- $\pm$ 0.1055, and for lot V. of the drones — "from the same hive" — is 2.2517 $\pm$ 0.1519. There is here a difference of 0.6880. The probable error of this difference is $\pm$ 0.1849. The standard deviation of lot I. of drones is 2.4023 $\pm$ 0.1620 and that of lot III., of the same sex, also from the same hive is, 2.9598 $\pm$ 0.1412. The difference here is 0.5575 $\pm$ 0.2149. This is due to the extremely small size of the series measured. Since the formula for the probable error of the standard deviation is 0.6745 (stand. dev./$\sqrt{2n}$), we see how rapidly an increase of "n" — the number of individuals measured — decreases the error of the result. But it is manifest that the differences between the two sexes, as shown by these data are of no significance; for, far within the probable error of our work, we get as great a difference between two lots of drones (one lot being twice as large as either of the two considered in the comparison of the different sexes) as we get between the two sexes.

I have not taken the time to go over the rest of Messrs. Casteel and Phillip's work; but, having reached the above results with the first and the last tables will leave them to go over the intervening ones. It is also probably unnecessary to remark that, even if it turns out that the greater variability of the drones can be established, their proofs of their theory to account for this difference seem rather unsatisfactory.

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THE SEXUAL ELEMENTS OF THE GIANT SALAMANDER, CRYPTOBRANCHUS ALLEGHENIENSIS.

ALBERT M. REESE.

In the spring and early summer of 1902, the author made strenuous efforts to obtain embryological material for investigating the development of the hellbender (*Cryptobranchus Alleghcniensis*). These unsuccessful efforts have been described in an article entitled "The Habits of the Giant Salamander." [1]

In the fall of 1903, another effort was made to obtain the desired material, and a dozen or more live hellbenders were obtained as the result of a trip to the region of the Allegheny River, in western Pennsylvania.

These animals were all about the same size, 45 cm. in length, and were sent by express from their native stream to Syracuse, N. Y.

Upon opening the box, in which they were shipped, after its arrival in Syracuse, a number of eggs were found scattered through the grass that had been placed there to protect the animals during their trip. There seemed to be no difference in the coloration of the males and females, and the only way in which they could be distinguished was by the fact that, in the males, the lips of the cloaca were considerably swollen by the enlargement of an elongated mass of glandular tissue on each side.

In handling one of the ripe females of this lot of hellbenders, the author was bitten on the thumb; this was the only time in which any attempt to bite had been noticed, though many dozen animals had been handled at many different times. The bite was not at all serious, being merely a painful pinch which scarcely broke the skin. In removing one of the females from the box in which they had arrived, an egg, enclosed in its jelly-like envelope, was seen protruding from the cloaca. By gently pulling this extruded egg, it was found that a whole string of eggs could be drawn from the cloaca, without apparently injuring them in the least.

[1] *Popular Science Monthly*, May, 1903.

Each egg is a spherical yellow body, about 6 mm. in diameter, resembling somewhat the yolk of a miniature hen's egg. It is surrounded by a clear gelatinous envelope, which is arranged in two distinct layers (Fig. 1).

When removed from the gelatinous envelopes, as may easily be done by cutting through the latter with a pair of fine scissors, the egg is seen to be enclosed in a very thin and delicate vitelline membrane which is easily torn in handling.

The yolk, which is apparently evenly distributed throughout the egg, is made up of a compact mass of granules of various sizes (Fig. 2).

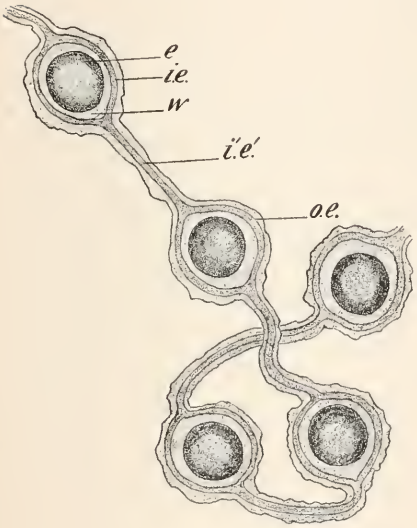

FIG. 1.

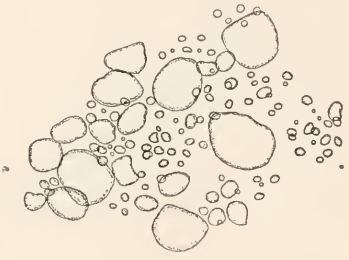

FIG. 2.

The egg is surrounded by a small amount of watery material (Fig. 1, *w*) which is, in turn, enclosed in a capsule of more dense jelly, the inner envelope (Fig. 1, *i. e.*). The inner envelope is continued as a solid, more or less tough cord of jelly (*i'. e'.*) from egg to egg, and binds them together in the continuous strings that have already been mentioned. The distance between two adjacent eggs of the string is usually about four or five times the diameter of the egg, but the elasticity of the jelly will, of course, permit the eggs to be drawn much further apart.

The outline of the inner envelope is sharp and even, while that of the outer envelope (Fig. 1, *o. e.*) is more or less irregular and uneven. The outer envelope is composed of such transparent jelly that it might easily be overlooked at the first glance. It forms a continuous layer over the entire mass of eggs.

When the unfertilized eggs are left for some days in water, they become very much swollen, by the osmosis of water through the vitelline membrane, and may eventually burst.

There was no apparent swelling of the gelatinous envelopes on coming in contact with water as is described in connection with some other amphibian eggs.

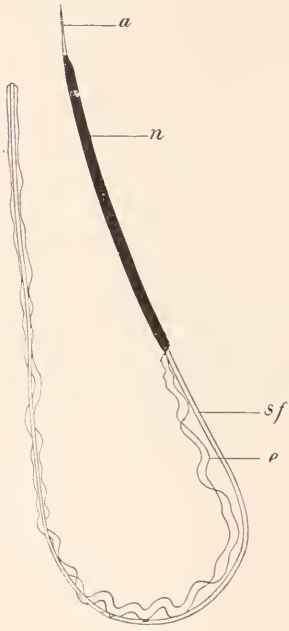

FIG. 3.

Several dozen eggs were obtained from one average-sized female, about two dozen being drawn, without apparent injury, from the cloaca, while the rest were obtained only after killing the animal and opening the body cavity. All the eggs obtained in the latter way were found to be contained in the right oviduct, the ova of the left ovary being nearly all in a very immature condition. Whether or not this was a normal condition, indicating perhaps, a very prolonged breeding season, it was not possible to say.

The spermatozoa were obtained as a milky fluid from the living males by the usual process of stripping, though considerable pressure had, in most cases, to be exerted. They were immediately examined under the higher powers of the microscope, but no motion could be detected, though it would naturally be expected that spermatozoa obtained in this way would show the usual activity of mature spermatozoa.

An attempt was made to artificially fertilize the eggs by putting them into a dish of water into which a great number of spermatozoa had been stirred, but the attempt was entirely unsuccessful.

No structures resembling spermatophores were discovered, and there was nothing that would seem to give any indication of the method by which the act of fertilization was accomplished.

A single spermatozoön, as seen under a magnification of about 1,300 diameters, is shown in Fig. 3.

Fairly good preparations were easily made by drying them rapidly on the slide, and staining in hæmatoxylin and eosin.

The nucleus, *n*, is very much elongated, so that it makes up almost one third of the entire length of the spermatozoön. It is capped, at its anterior end, by a sharp, gradually-tapering apical body, *a*, which is plainly differentiated from the nucleus proper by the fact that it does not take up the stain to any great extent. No structural details in the nucleus or apical body can be discerned with the magnification used, nor is any middle-piece distinguishable. The tail, which is comparatively stout, consists of a central supporting fiber, *s. f.*, which takes up the stain slightly, surrounded by a transparent envelope, *e*, which does not stain at all. The envelope is usually considerably wrinkled and twisted, probably by the rough method of fixation.

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THE RHYTHM OF IMMUNITY AND SUSCEPTIBILITY OF FERTILIZED SEA-URCHIN EGGS TO ETHER, TO HCl, AND TO SOME SALTS.

E. G. SPAULDING.

Introduction.

The experiments described in this paper were undertaken during the summer of 1902 at the Marine Biological Laboratory upon the suggestion of Dr. A. P. Mathews; their publication has been delayed because of the pressure of other work and of the desire to, if possible, get beyond their mere description to their meaning. This end is believed to have been attained in connection with the working out of a synthesis of the artificial parthenogenetic methods, the detailed results of which attempt appear in a preceding paper.[1] The effectiveness of all these methods and so the special physico-chemical result of normal fertilization and the nature of cleavage processes can, it is believed, be explained from a unitary standpoint, viz.: if it is considered that in the process of cleavage an average decrease in surface tension takes place as a result of the equilibrating of a potential difference between osmotic pressure and surface tension, accompanied by such electrolytic changes as cause the constricted form. That this average decrease in surface tension takes place is a necessary inference from the change from the approximately spherical to the constricted form of the egg at cleavage, for this means an increase in surface. It carries with it, therefore, the decrease in that potential, osmotic, which opposes surface tension in direction. The preceding cause in these events is the creation of a potential difference by first increasing the osmotic pressure, which is done artificially by each of the parthenogenetic methods. Accordingly with the equalization of this difference, caused, *e. g.*, by a splitting up of colloidal particles or molecules, there is in the case of eggs of marine forms an absorption of water. Both of these

[1] Spaulding, E. G. "The special physics of segmentation as shown by the synthesis, from the standpoint of universally valid dynamic principles, of all the artificial parthenogenetic methods." Biological Bulletin, February, 1904.

last events either alone or together may constitute what is termed liquefaction.

The results of the experiments given below can, it is believed, be interpreted in agreement with this view of segmentation. They were undertaken primarily as an extension of experimental work which had already been done on the action of various chemical compounds on protoplasmic bodies, but were limited to the study of such action on the eggs of *Arbacia* at successive periods after fertilization.

The existence of a rhythm of immunity and susceptibility has been shown already by Lyon in studying the effect of KCN and of lack of oxygen upon the fertilized eggs and embryos of the same form,[1] and it has been found also that many eggs do not segment at all in the absence of oxygen, notably *Arbacia*[2] and *Ctenolabrus*.[3] Lyon also found this summer that *Arbacia* eggs required more oxygen during precleavage and gave off more CO_2 during cleavage than at other times.[5]

From these results it may be inferred in analogy to a large number of instances well known in chemistry that at least the *ultimate* effect of oxygen on the processes conditioning cleavage is the causing of analytic chemical changes; *i. e.*, fermentation, one might say, occurs and CO_2 is given off, as Lyon found. Previous to this, however, synthetic processes may take place which in turn as certain preferments become active[4] give rise to molecular splitting. The result of such analytic change is that increase in osmotic pressure and therefore the creation of that potential difference between it and the surface tension which we have found to be necessary and in the equalization of which both potentials decrease, water is absorbed, and the egg cleaves.

The hypothesis to be deduced from this and which might serve as a guide in our experimentation is that any method either (1) of preventing this necessary preliminary increase in osmotic pressure, or of compensating it after it has been created, or (2) of increasing it beyond a certain point, will tend to do away with

[1] Lyon, E. P., *American Journal of Physiology*, VII., 1.

[2] Lyon, *loc. cit.*

[3] Loeb, J., *Archiv für die gesammte Physiologie*, 1895, LXII.

[4] Cf. Hofmeister, "Chemische Organisation der Zelle."

[5] Personal communication.

the event of cleavage. To the first two possibilities correspond the effect respectively of lack of oxygen and the use of strongly hypertonic solutions on the fertilized egg; to the second method our own results with ether, HCl, etc. From this hypothesis it can also be reasonably inferred that the nearer to the point of termination of the preparatory process that either method is used, so much the less will its effect in general be, and this supposition is again confirmed by experimental results.

Lyon[1] found that the effect of KCN on the fertilized *Arbacia* egg, taking, *c. g.*, various strengths of a titrated solution mixed with sea water, was the indication of "successive stages of relatively high and low resistance in each cleavage." Putting the eggs into the solution at successive five-minute periods after fertilization and allowing them to remain perhaps one hour, then washing and removing to sea water, he found that "there is a stage about ten or fifteen minutes after fertilization when the egg is especially susceptible to KNC." "Again *soon after* the first cleavage comes a second stage of small resistance; a third follows the second division." "The resistance of the egg to KNC increases up to a *maximum* up to the time of *separation into* the two cells." "The effect of KNC is the same as lack of oxygen."

In interpretation of these results Lyon says that the processes dependent upon oxygen seem to begin about 10–15 minutes after fertilization, for if they are inhibited the egg does not segment and they recur at each segmentation. To identify them with the morphological processes of the splitting and separation of the chromosomes or with the dissolution of the nuclear membrane seems to him to be impossible, for these occur too late to be directly affected. Wilson and Matthews,[2] he says, mention however two processes which occur sufficiently near to the susceptible stage to be worthy of consideration in this respect. One is the growth and division of the sperm aster, the other the growth of the nucleus. From the part which in order to explain the constricted form of cleavage[3] must be attributed to, as played by each of these processes, the supposition that they are affected by a lack of oxygen, by KCN, etc., receives confirmatory evidence.

[1] *Loc. cit.*

[2] *Journal of Morphology*, 1895, X., p. 319.

[3] Lillie, R. S., Biological Bulletin, IV., March, 1903; and *Am. Jour. of Physiology*, VIII., 4, Jan. 1903.

But furthermore, whatever the morphological elements may be, it must also be admitted that in the processes leading up to and culminating in cleavage, we are dealing with chemical and electrolytic and consequently also with osmotic phenomena, coexisting with those of surface tension. That these first two which condition the other two are, however, not uniform, but, rather, are varying, *i. e.*, rhythmical, during that period must be admitted to explain the observed rhythm in morphological changes.

The experiments herein described serve the purpose then of testing the above-mentioned hypothesis of the existence of a liquefaction during the event of cleavage, and of a rhythm of increasing immunity up to and of marked *susceptibility during* that time. To this end use was made of ethyl ether, HCl, KCl, NaCl and sodium citrate solutions.

Theories of the Nature of their Action.

From the position that has been taken in this and a previous paper that, inasmuch as in protoplasm we are dealing with colloidal (probably also electrolytic) particles in solution, we therefore in segmentation necessarily have to do ultimately with the relations of two kinds of energy, osmotic and surface, and that the cleavage process itself depends upon the existence of an uncompensated potential difference between these, from this it follows that this potential difference might be caused *in either of two ways*, viz., at the same time that either one is kept constant, by changing the other, *i. e.*, either increasing the osmotic or decreasing the tension factor, the former being identical with the energy of the particles in solution, the latter with that of the solvent. The theories also which we find advanced in order to explain the nature of *stimulation* seems to us to be in complete agreement with this view. For example, we find the statement that "stimulation consists in the precipitation, *i. e.*, gelation, of colloidal particles and is due in the case of positively charged particles to the negative ions, *i. e.*, to the charge;[1] the inhibition of this stimulation, *i. e.*, what in some cases is termed poisoning, to the positive ions"; for negative particles the converse would hold

[1] Mathews, A. P., "The Nature of Nerve Stimulation, etc.," *Science*, March 28, 1902.

true. In any case the osmotic pressure would necessarily be affected. Both the stimulating and poisoning effect have furthermore been correlated with the valency.[1] In the case of a compound of anion and kation, both of which are monovalent, like NaCl which does and KCl which does not stimulate easily and when in both therefore the charges might seem to offset each other, the stimulating effect, *c. g.*, on the nerve has nevertheless been said to be due to the "overbalancing" of the kation by the anion, and conversely for the inhibitory effect. This of course is not real explanation unless the difference in effect can be correlated with a difference in some such quality as velocity of diffusion or solution tension, and Mathews has this summer shown that the poisoning qualities of the metals and non-metals as well are in fact a function of this latter. Some ground for this "overbalancing" effect seems to be furnished by the fact that in the case in which *c. g.*, a divalent anion is combined with a monovalent kation (2) a greater stimulating effect is observed. Thus KCl does not stimulate the nerve at its osmotic pressure, K_2SO_4 does occasionally, K_3 citrate stimulates in solutions of a gram molecule to 22,000 c.c. H_2O. But even here the number of opposing charges is the same. The kations therefore differ in some way other than in their mere number of charges. This must also hold true of the anions because of the increasing stimulating effect on the nerve of NaCl, NaBr, NaI and NaFl. The suggestion has been made that the difference in effect when the charges are the same in number is due to a difference in the translatory path of the charge around the atom; but as a cause for this latter difference must in turn be assigned the admission of an ultimate difference in the atoms themselves would seem to be necessitated.

While the salts therefore seem to affect the colloidal particles directly, the known inhibitory action of the anæsthetics would accordingly have to be identified with a direct effect on the solvent and so only indirectly on the solute. Thus it may be considered that the anæsthetics as being better solvents in most cases than water have the same effect on colloidal particles as do like charges, which repel; therefore they increase the osmotic

[1] Loeb, *Archiv für die gesch. Physiologie*, Bd. 88.

pressure. It is believed that on this basis the varying effect of ether on the eggs of *Arbacia* at successive periods after fertilization can be explained.

Experimental.

1. *The Effect of Ethyl Ether.*

The general methods of experimentation may be outlined as follows: First, a slightly supersaturated solution of ether in sea water was prepared, kept tightly corked, and when used a portion was drawn from the bottom, thus ensuring a saturated solution. The eggs were fertilized in the usual way, good lots from among a number being selected. At successive periods these eggs were transferred to staining jars containing 50 c.c. of the solution used. The strength of the solution actually used was accurately controlled by starting with twice that strength and then diluting exactly one half, in part with the sea water necessary for transferring purposes. The eggs were allowed to stand in these *covered* jars for the length of time selected; the solution was then carefully drawn off; the eggs were thoroughly washed twice with sea water, which was again added, and given time to develop. In important experiments the lots were each observed twice. All the experiments were conducted at the room temperature, about 20° C.

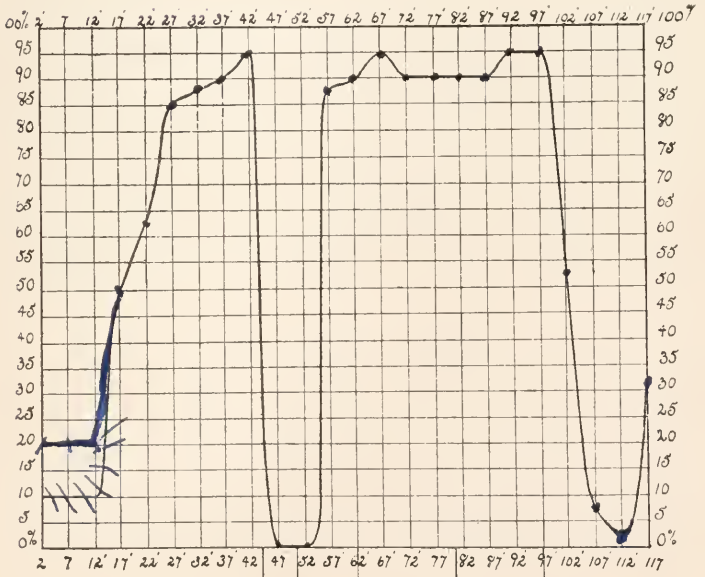
The practical problem presented was to get such a strength of solution and time of exposure that of the eggs transferred at the various periods after fertilization some would be stopped in their development, others not. For each value of the one factor there would probably be a corresponding value of the other, the product being a constant. An abstract record of what were essentially preliminary experiments is given on the next page.

From these results it seemed that the correct relation between the two variables, time and solution strength, had been found; accordingly in the next experiment a one sixty-fourth saturated ether solution was used for 25′, which gave very satisfactory results. These are tabulated and plotted on page 008.

In the plotting of the curve of these results the abscissæ represent the times of transferral after fertilization, the ordinates the per cent. of swimmers found by as accurate observation as possible.

Exper.	Strength of Solution.	Period After Fertilization.	Time of Exposure.	Observation.	Control.
I. July 24.	Saturated.	Every 15′. 8 lots.	1 hour.	All dead in the stage of treatment.	Well developed
III. July 26.	$\frac{1}{4}$ and $\frac{1}{8}$ sat. sol. by diluting with sea water (same lot of eggs).	Just before and after each cleavage.	Various.	All dead.	Well developed
IV., A. July 29.	$\frac{1}{16}$ sat. sol.; one lot of eggs for A, B, and C.	At "critical" periods, *i. e.*, just before and after each cleavage, as below in B.	30′	All dead.	Control good, regular.
IV., B.	$\frac{1}{32}$ sat sol. Lot (1).	15 minutes. (Lyon's 1st critical point.)	45′	50% unsegmented, but pigmented and swollen, some in 4 and 8 cell stage; 20% swimming.	"
	(2)	50′, 1st. cleavage just beginning.	30′	80% dead, swollen, pigmented, 20% swimming.	"
	(3)	1 hr. 10′, toward end of cleavage.	30′	87% dead, irreg. segment., some in 16 cell stage 13% swimming.	Segmentation going on.
	(4)	2 hrs., during 2d. cleavage.	30′	All stopped in 4 cell stage.	Control good.
	(5)	2 hrs. 40′, after 2d. cleavage.	30′	All dead.	
IV., C.	$\frac{1}{64}$ sat. sol. (1)	15′, Lyon's critical point.	45′	8 hrs. afterward all had segmented, 8, 16, 32 cell stage; 25% swimming.	Control good.
	(2)	50′, cleavage just beginning.	30′	(Like C 1.)	
	(3)	70′, toward end of cleavage.	30′	Nearly all swimming.	
	(4)	2 hrs., during 2d. cleavage.	30′	2–32 cell stages present; decomposed and pigmented; $\frac{1}{15}$ swimming.	
	(5)	2 hrs. 40′, after 2d. cleavage.	30′	$\frac{1}{5}$ swimming.	

Exper. V. July 30.	Solution 1/64 Sat.	Time After Fertiliza-tion.	Exposed to Solution.	Observation.	Control.
Lot 1		2′	25′	20% swimming.	
" 2		7′		20 "	
" 3		12′		20 "	
" 4		17′		50 "	
" 5		22′		62½ "	
" 6		27′		85 "	
" 7		32′		88 "	
" 8		37′		90 "	10% in 2-cell stage, 44′ after fertilization.
" 9		42′		95 "	
" 10		47′		0 "	20% segmented.
" 11		52′		all dead, pigmented, and swollen.	20% "
" 12		57′		87.5% swimming.	
" 13		62′		90 "	90% "
" 14		67′		95 "	" "
" 15		72′		90 "	
" 16		77′		90 "	
" 17		82′		90 "	95% "
" 18		87′		90 "	
" 19		92′		95 "	
" 20		97′		95 "	33% in 4-cell stage.
" 21		102′		55 "	
" 22		107′		7.5 "	
" 23		112′		2 "	
" 24		117′		30+ "	



The character of this rather remarkable curve is obvious. Up to within twelve minutes after fertilization the resistance remains the same, but from this point on it gradually rises up to either *just before* or the *beginning* of the first cleavage; during the early part of cleavage it falls to zero, with a sharp rise afterwards and a fall at the second segmentation.

The more important question however is to get at its meaning. To get at this we take, corresponding to the general rise in immunity up to the time that cleavage is beginning, the greater demand for oxygen, established by Lyon in his work this summer. This might mean in view of the fact that either at least just preceding or *for some time* before cleavage an increase in osmotic pressure must take place, as we have shown, either one of two things to account for this, viz., either that the oxidation process is at first synthetic and subsequently determines analytic events; or that it is analytic from the start. Also to be correlated with this is the known effect of ether as a better solvent than is water. This is identical with its causing a greater degree of solution and consequently an increased osmotic pressure. The inhibiting effect of the ether on the eggs at the critical period in the above curve may be ascribed then, we believe, to its *augmentation* of the normal predominence in osmotic pressure at that time, *i. e.*, to its increase of that difference of potential in the direction of pressure-tension *necessary* for cleavage. Accordingly in the equalization of this *augmented* potential difference the eggs would be expected to increase in size more than usual in their attempt to divide, and this is confirmed by the *observed swollen* appearance, even when as in some cases division takes place once and then stops.

This increasing immunity up to the maximum can be explained then in two possible ways. If synthetic as simple oxidation processes procede the analytic then during that period there is something to *oppose* the dissolving effect of the ether; but since this *opposition* would seem to exist *equally* all through the precleavage period, the *rise* in immunity would be hard to account for in this way. On the other hand if analytic processes take place from the start as a result of the use of oxygen then the *longer before* cleavage that the exposure to ether is made the *greater* should be

its effect in augmenting the normally occurring increase in the pressure, and the point of greatest susceptibility would be at such points and also at that of the normal maximum pressure, viz., just before or during cleavage. This explanation therefore accords best with the sharp rise in the curve, and is supported also by the evidence from the parthenogenetic methods for *Arbacia*, in which the pressure is first increased, *sometime before* the cleavage, which takes place after the return from the hypertonic solution to the sea water.

The characteristics of the above curve were confirmed in general by *Experiment IV.*, B and C, already presented, and more especially by three subsequent experiments, as can be seen from the following records:

EXPERIMENT VII.

August 22, 1/64 sat. sol. Time of exposure, 1/2 hr.

Lot.	Period After Fertilization.	Observation.	Control.
1	3′	66% unsegmented.	
2	19′	90% living.	
3	31′	90% "	
4	56′	95% dead, many in 2-cell stage, pigmented, swollen.	Middle point of segmentation.
5	63′	25% swimming.	
6	79′	25% "	
7	96′	All dead in 4-cell stage.	During second cleavage.
8	112′	" "	" " "

Experiments VIII. and IX., August 23 and 29. Solution, one sixty-fourth saturated ether, one half hour exposure. Lot 1, one half hour after fertilization all living. Lot 2, during segmentation, *all dead* in 2-cell stage.

EXPERIMENTS WITH HCl.

Preliminary and theoretical. According to the views that we have previously discussed the hydrogen ion in the case of the nerve is held to inhibit the stimulating action of the chlorine ion, "overbalancing" this more than do either K, Li, NH_4 or Na. This may be due to the greater velocity of diffusion of H, which is 325, that of Cl 70.2 at 25° C.[1] On the other hand it has been supposed that the H ion brings about the parthenogenetic devel-

[1] Ostwald.

opment in *Asterias*. These two seemingly contradictory effects cannot, however, be so in reality and the difficulty may be done away with if it is borne in mind that the effect depends as much on the character (electronic) of the colloid as on the agent. For on positively charged particles the H ion would have a repelling, *i. e.*, dissolving ; on negatively charged, the opposite effect.

If all the protoplasm of the *Arbacia* egg was uniformly positive just prior to or during segmentation it accordingly might be deduced that H ions would have the same effect in increasing the osmotic pressure as does ether, and so of inhibiting development. Lillie,[1] however, has shown that at cleavage the cytoplasm is markedly electropositive, the nucleus negative. Accordingly at that time he holds that the periphery repels the free kations within the egg and the nucleus the anions, so that the kations then predominate at the center, the anions at the periphery. As like charges repel each other, this is made to account for the constricted form of the egg at cleavage. In agreement with this view the effectiveness of the H ions, in parthenogenetic methods, *in the environment* might be considered to be due to their induction of a predominance of negative charges at the surface and this in turn of positive charges at the astral centers. If this be so, however, then other kations ought to have the same effect ; but they do not. This indicates a specific action by the H ion, which it might have in accordance with its high diffusion velocity. It alone might therefore be considered to penetrate the egg membrane because of its and the latter's definite chemical make-up ; yet there remain difficulties even here in explaining why it should do this, since, if, at least before cleavage, the membrane and cytoplasm are themselves positive they would tend to repel the H ions rather than to attract them. Only provided the surface were negative from the start could the attraction be explained. If, however, the egg, when *just about* to divide, were put into such a medium of H ions, it is reasonable to suppose that since the surface at least then is negative these might be attracted ; but again it is difficult to understand how they can go further, since the cytoplasm is even yet positive. However, if they succeed

[1] Lillie, R. S., *Am. Jour. of Physiology*, VIII., IV., and BIOLOGICAL BULLETIN, IV., 4.

in acting on the cytoplasm the effect would be a repulsion of its particles and an increase in pressure, the same as that of ether. Accordingly it would *a priori* be probable that the period of least immunity to HCl would coincide with that of the greatest normal pressure, viz., just before cleavage.

Experimental.

Two preliminary experiments with seventeen different strengths showed that the proper strength of solution, for an exposure of one half hour, was between a $\frac{1}{400}$ and $\frac{1}{500}$ normal HCl soluion. Accordingly a $\frac{1}{450}$ *n* solution was next tried with the following results:

Experiment IV.

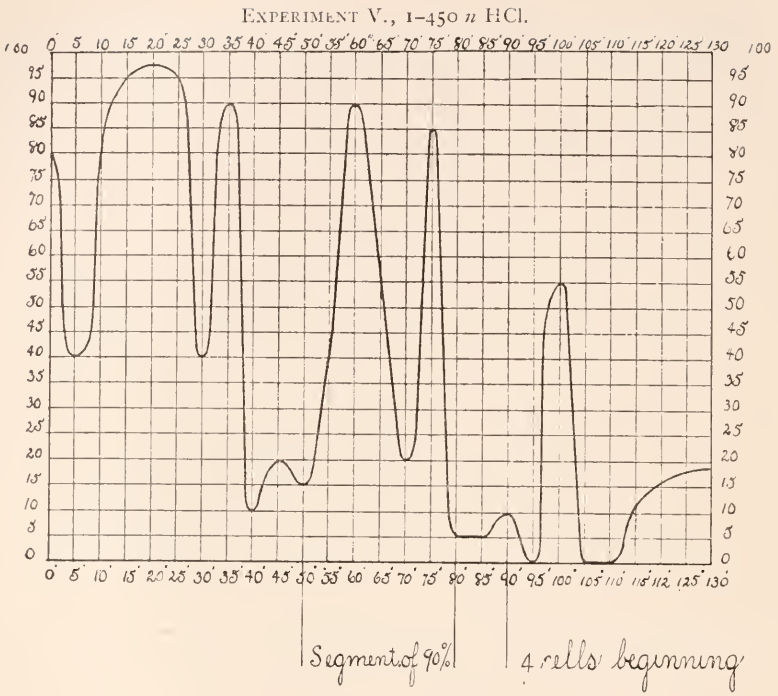
August 3, 1.450 *n* HCl solution (by diluting with sea water); three series, 25′, 30′ and 35′ exposure.

Lot	Time After Fertilization.	Series A, 25′. Per Cent.	Series B, 30′. Per Cent.	Series C, 35′. Per Cent.	Control. Per Cent.
1	1′	No segmentation at all.[1]	A few swimmers.	A few swimmers.	
2	16′	50 swimming. 30 blastulæ, stopped.	80 swimming.	50 swimming. Gastrulæ.	
3	31′	*Quite undeveloped and disintegrated.*	Like A^3.	Like A^3.	
4	41′	80 swimming.	40 swimming.	40 swimming.	Segmentation began here.
5	46′	*All dead in 1- and 2-cell stage.*	Like A^5.	Like A^5.	Segmentation going on.
6	53′	40 swimming.	20 swimming.	20 swimming.	Toward end of cleavage.
7	59′	50 "	50 "	50 "	
8	77′	*All dead in 2- and 4-cell period.*	Like A^8.	Like A^8.	15 were in 4-cell stage.

August 5, *Experiment V.*, $\frac{1}{450}$ *n* HCl solution, used every five minutes for one half hour. The results are plotted on the next page, the times after fertilization being the abscissæ, the percentage of swimmers the ordinates, and are in general confirmatory of the previous experiments with HCl.

Two other experiments were made with HCl, which gave a general confirmation of the two described. These all agree in giving a rise in immunity soon after fertilization, then a fall at

[1] The difference between A^1, B^1, and C^1 may be due to the effect of the necessary manipulation on the eggs transferred so soon after fertilization, viz. A^1; B^1 and C^1 were necessarily transferred a little later. Cf. Mathews, *American Journal of Physiology*, VIII., IV., "Importance of Mechanical Shock on Protoplasmic Activity."



30′, followed by a second rise and a second fall respectively before and during segmentation. This fall is repeated at the beginning of the second cleavage.

Comparison of this with the 'ether curve' however shows a difference in respect to this 'thirty-minute fall,' and that this should be so is to be expected from the possibility of the agent here used (HCl) directly affecting the colloidal particles as well chemically as electrically, as the evidence for a specific H ion action indeed indicates; while in the case of ether the solvent alone is first concerned.

Assuming that the H ions in some way penetrate the egg, the increase in osmotic pressure resulting therefrom might be connected with the dissolution of the nuclear membrane, which occurs at about the time of the first, the 30′ fall,[1] in such a way that an abnormal increase in pressure results from both, which inhibits development. This however could not be due alone to the electronic action of the ions for were this so ether also

[1] Mathews and Wilson, *loc. cit.*; cited by Lyon.

would have the same effect in increasing the pressure abnormally and causing a fall in immunity at the same time, which it does not do. Hence from this, together with the evidence, in other instances, of H acting differently than do other ions, and the probability that the dissolution of the nuclear membrane is caused or accompanied by chemical changes, it may be inferred that the H ions have a specific chemical effect, which, in addition to their electronic action, accounts for the fall in immunity at about 30′ after fertilization.

Both series of experiments agree, however, in making the period of greatest susceptibility just at the beginning and during the earlier part of cleavage, though for the HCl this drop seems to come somewhat earlier than for ether. The explanation for this effect of HCl we believe to be essentially the same as for that of ether, viz., that the H ions in some way penetrating the membrane cause a repulsion of at least the cytoplasmic particles, thereby *augmenting* the normally increased pressure to such an extent that further development is inhibited.

Experiments with KCl, NaCl and Na citrate.

The acceptance of a specific action for the ions is, of course, by implication not limited to H, but is quite as necessary for others, like K, Na, Ca, etc. For instance Loeb in one place holds that the kations (specific) and not the anions are poisonous,[1] for the reason that the newly fertilized *Fundulus* egg will develop in KCl but not in NaCl [2] and because, of fertilized *Arbacia* eggs in $\frac{5}{8}n$ NaCl only 10, 20, and in one case 50 per cent. began to segment, the majority stopping in the 2-cell stage, while in $\frac{5}{8}n$ KCl, 70–80 per cent. segment to 8 cells.[3] Opposed to this view of Loeb's is that which ascribes toxic effects also to the anion, for, *e. g.*, NaCl, NaBr, NaI and NaFl have a different poisoning effect. The two views, however, are quite compatible if the colloids affected are in the two cases of different sign. Loeb also finds that the toxic effects of Na salts is a function of the valency, increasing from the acetate to the citrate.[4] Further-

[1] Loeb, *Am. Jour. of Physiology*, III., VII. and VI., VI. and *Archiv für d. ges. Physiol.*, Bd. 88, 1901.

[2] Loeb, *Am. Jour. of Physiology*, III., VIII.

[3] Loeb, *Am. Jour. of Physiology*, III., IX.

[4] *Am. Jour. of Physiology*, VI., VI.

more, as an illustration of the lack of a consistent theory here, are the views of Loeb, that the antitoxic as well as toxic effect is a function of specific kations, and of others that where the kations are toxic the anions are antitoxic. For instance, in 100 c.c. $\frac{5}{8}n$ NaCl + 8 c.c. $\frac{1}{64}n$ $CaSO_4$ or $Ca(NO_3)_2$ 70 per cent. of *Fundulus* eggs develop, while if Na_2SO_4 be substituted they do not. Ca is therefore considered to be antitoxic to Na. Al_2Cl_3 and $Cr_2(SO_4)_3$ also have the same inhibitory effect on Na, but in smaller quantities. Loeb therefore concludes that the toxic and antitoxic effect of the ions is a function of their valence, but that only kations are poisonous. The necessity for such "balanced" solutions also holds good according to Loeb for muscle and for the contractions of *Gonionemus;* "margin and center must contain three ions, Na, K and Ca."[1]

The opposite view is that in the instance of, *e. g.*, NaCl poisoning but KCl not, the difference in effect is due to a greater overbalancing by the Cl ion in one case than in the other.[2] The effect is due in any case to both the ions. Both may be either toxic or antitoxic according as the colloid is like or unlike in charge and the normal event is one of gelation or of liquefaction.

Accordingly if the normal progress of cleavage in *Arbacia* demands liquefaction, *i. e.*, increased pressure and absorption of water, any salt either preventing this by unlike charges or augmenting it by like beyond a certain point may be assumed to interfere with or inhibit division. And from the data at hand we may expect NaCl to do this to a less degree than KCl.

Experiment IV., 7 pts., $\frac{5}{8}n$ KCl sol. to 2 of sea water, the other conditions as in III., gave *exactly the same* results, and is tabulated on the next page.

Examination of this record makes manifest a decrease in immunity beginning 15 minutes before cleavage, much as with HCl, but reaching its *maximum during segmentation.* The same *explanation* of this effect that was made for HCl and ether holds good, we believe, here; but the thirty-minute fall obtained with HCl is not present in these series, which indicates a different specific chemical effect of H and of K ions. Two subsequent KCl experiments confirmed these results.

[1] *Am. Jour. of Physiology*, III., VII.

[2] Mathews, *Science*, March 28, 1902, and May 8, 1903.

EXPERIMENTS WITH KCl.

	Solution.	After Fertilization.	Exposure.	Observation.	Control.
Exper. I., Aug. 16.	$\frac{1}{3}n$.				
Lots I.–III.		At *critical* periods before cleavage.	1 hour.	90-95 % swimming.	Good.
Lot IV.		During cleavage, 66′ after fertilization.	1 "	75 % swimming.	
Exper. II., Aug. 17.	$\frac{5}{12}n$ KCl.		1 hour.		Good.
Lots I.–IV.		Before cleavage.		All dead in 2 cells.	
Lot. V.		During cleavage.		Dead.	
Exper. III., Aug. 19.	8 pts. $\frac{5}{8}n$ KCl + 2 of sea H_2O in transferring.	every 5′	1 hour.		
Lot I.		5′		80 % swimming.	80 % swimming.
" II.–VIII.		(10′–40′)		80 "	
" IX.		45′		70 "	
" X.		50′		60 "	
" XI.		55′		50 "	
" XII.		60′		25 " Remainder dead and disintegrated.	Segmentation began here, 60′ after.
" XIII. and XIV.		65′–75′		5–10 % swimming, remainder pigmented and disintegrated.	

Experiment I., August 29, with NaCl, 15 pts. *n* to 1 of sea water, exposure one hour, at chosen critical periods. Observation showed a slight rise in immunity from 70 to 95 per cent. up to the beginning of segmentation, then a fall to 70 per cent. during that process, but as opportunity for further experimentation was lacking this result is hardly conclusive, though by itself it is confirmatory of the supposed greater immunity to NaCl than to KCl.

Experiment I., with the Na citrate, August 13; 3 series, viz., 2 c.c. of seven-fourths *n* Na citrate to 300, 400, 500 c.c. respectively of sea water, *diluted* one half at transferral. 15′ periods, 1 hour exposure. Parallel observations showed that the eggs so treated were *ahead* of control both in beginning to segment

and in the number segmented at all cleavages. No period of susceptibility was indicated. This acceleration effect might according to the views above discussed be ascribed to the so-called "overbalancing effect" of the trivalent radical, but the experiment was not repeated because of lack of time.

Summary and Conclusions.

1. There is a pronounced *rise* in *immunity* of fertilized sea-urchin eggs to *ether* up to either *just before* or the *beginning* of segmentation; the exact point is impossible to determine owing to the unevenness of the cleavage. A *sharp decrease* then occurs, followed by a *sharp rise toward the end* of the cleavage. A repetition of this occurs at the second segmentation.

2. *Similar* changes are found resulting from the use of HCl, KCl, and NaCl with the difference that the fall in immunity comes somewhat earlier with KCl than with HCl and with this than with ether. All of these differ therefore from Lyon's interpretation of the susceptible points with KNC as occurring "after division," nor is a 15′ period found, although with HCl there is a fall in immunity at 30′ after fertilization.

3. The marked decrease in immunity "at cleavage" caused by the four agents employed seems to be explainable on the basis that all, in one way or another, *augment* beyond a certain point the increase in osmotic pressure normally necessary for cleavage. The results obtained seem therefore to be confirmatory of the position presented by the author elsewhere[1] that cleavage is due to the equalization (Ausgleichung und Umformung) of an uncompensated potential difference between osmotic pressure and surface tension, accompanied by electronic phenomena which cause constriction.

The author wishes to acknowledge his appreciation of the many opportunities offered him by the Laboratory and of the kind advice and suggestions of Dr. Mathews.

College of the City of New York,
Department of Philosophy, January, 1904.

[1] Biological Bulletin, February, 1904.

BUDDING TENTACLES OF GONIONEMUS.

GEORGE T. HARGITT.

While looking over specimens of *Gonionemus* for class work, and being somewhat on the lookout for any cases of variation, etc., my attention was arrested by an unusual appearance of a tentacle. Near the distal end was present a small knob which at first glance appeared simply as a protuberance, apparently without any very definite form or structure.

On further and more careful examination it seemed to me to warrant a careful study. It was somewhat similar to some of the conditions found by Hargitt[1] in his work on the variation of

FIG. 1.

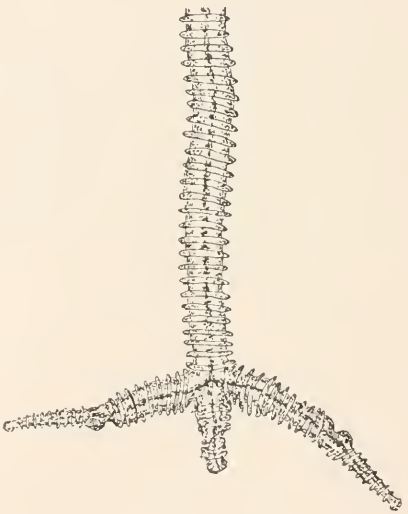


FIG. 2.

this form. The conditions referred to are the presence of bifurcated tentacles. He found a number of tentacles which had double, or in one case triple tips. In all these cases the extra tip or bud seemed to arise either from the suctorial pad or else immediately proximal to it. Fig. 1 represents a rather typical example of bifid tentacle. The specimen is one I found in C.

[1] "Variation among Hydromedusæ," BIOL. BULL., Vol. II., No. 5, 1901.

W. Hargitt's collection, but which he did not use in his paper on variation. The extra tip is seen to be much shorter than the main tip. Each tip is supplied with one of the suctorial pads, but the shorter one arises from a point considerably proximal to the pad on the main tentacle and directly from the tissue of the tentacle. No sign of injury is present. The appearance of this tentacle suggests the probable result of further growth of the bud shown in Fig. 3, except of course the lack of the pad at the base of the bud. Fig. 2, showing a trifid tentacle, is taken from Hargitt's paper on variation (*op. cit.*). It shows two branches arising from near the end of the main tentacle which seems to be degenerate as mentioned later. The buds here do not seem to arise from the suctorial pad of the main tentacle, which is not shown, but each bud is supplied with a pad near its tip. The knob on the tentacle under consideration, however, had more the appearance of a bud than a bifurcation. This was due chiefly to its small size which rather suggested that it was a very early stage in the formation of an extra tip to the tentacle.

The bud arose from a definite base which presented almost exactly the same external appearance as the normal suctorial pad. This similarity consisted not only in the smooth appearance, due to the absence of the ectodermal ridges found on the other parts of the tentacle, but also in its concave form, and the further presence of a bend or "knee" in the tentacle at this point; all of which are characteristic of the normal suctorial pad (Figs. 3–5). The bud arose from a depression in the base (Fig. 4) due to the cup-like shape of the pad already mentioned.

No external sign of injury was found either in this pad or in the surrounding tissue. The pad was of course not functional as an adhesive organ, another functional one being present nearer the distal end of the tentacle (Figs. 3 and 5). Whether this new pad formed after the beginning of the development of the bud from the old pad, whose functional activity would thus be destroyed; or whether a second pad formed first, and a bud began to develop from the old one (which would not then be necessary) simply as a result of the capacity for regeneration, or rather duplication of parts, inherent in the tentacles, is an extremely interesting question. Of course no direct answer can be

made without a considerable body of facts before us, coming from actual observations on this particular point; facts which it would be very difficult if not impossible to obtain. It seems to me, however, that we can suggest a probable answer from conditions observed in other cases, which bear more or less directly on this point. A comparison of Figs. 1 and 2 with some of those in Hargitt's paper show that buds do not always arise from pads. Indeed of the six figures of bifid and trifid tentacles shown in that paper and in this, only two show the bud as arising from the pad. This alone would show quite conclusively that there is no necessary or regular connection between the bud

FIG. 3.

FIG. 4.

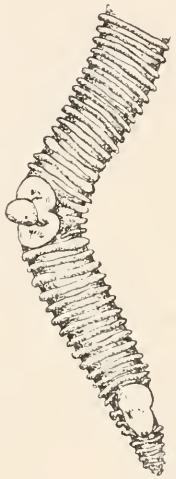


FIG. 5.

and the suctorial pad. This is further emphasized by the fact that I found nine tentacles having each three pads, apparently well developed and functional, and yet there was not the least sign of buds arising from any of the pads. These facts all point directly toward an unusual predisposition to a duplication of organs, and this perhaps offers the most satisfactory explanation of the budding and bifurcation of tentacles.

The bud showed a constriction near its middle region, but did not present externally any signs of annulation, or rather of the ectodermal ridges present on the old tentacle. However, nematocysts were present in abundance. The general appearance of the

bud is similar to that of the old tentacle, with the exception noted above, and is undoubtedly of the same structure.

Hargitt (*op. cit.*, p. 244) in referring to bifid and trifid tentacles suggested that they might have orginataed is the result of some injury to the distal end of the tentacle. This seemed to be especially indicated by the one trifid tentacle found. In this case there seemed to be a degeneration or atrophy of the median branch, which was probably the end of the original tentacle. From the sides of this tentacle two branches arose opposite each other which were considerably longer than the median tip (*cf.* Fig. 2). He says concerning the cause of this : "The degenerating middle tip would very naturally suggest the probability that an injury might have been the predisposing cause of the secondary tips ; on the other hand, it must not be overlooked that in each of the other specimens with double tips no such cause seems at all evident."

It was with the thought of trying to determine whether there was any sign of injury which might have influenced the formation of a bud in that region, as well as to determine the histogenic changes involved in its formation, that I was led to undertake a careful study of this budding tentacle.

The tentacle was stained in toto with borax carmine. Sections were cut transversely across the tentacle, thus making the sections of the bud longitudinal.

Fig. 6 represents a section of the entire tentacle showing the bud in its general relations. The entoderm of the bud is seen to be directly continuous with the entoderm of the tentacle. The bud is solid with the exception of a cavity at the distal end and there is no connection between this cavity and the cavity of the tentacle. It will be noticed, however, that the cells are arranged more or less definitely in two rows with the dividing line quite distinctly marked in the proximal region, as though in further growth these would pull apart and thus connect the distal cavity of the bud with the cavity of the tentacle. On either side of the bud are masses of the rather dense tissue which makes up the suctorial or adhesive pad already mentioned. This tissue resembles very much muscular tissue rather than glandular tissue, suggesting that the pad acts by virtue of its muscularity, rather than by

means of a secreted adhesive substance as suggested by Perkins.[1] At the edges of this pad is present the collar-like expansion of the ectoderm which is characteristic of this structure normally. Indeed, the shape, size and contents of these cells, as well as their method of staining and general appearance, is almost exactly the same as in the normal pad. Thus there seems little doubt that there was originally present here a functional suctorial pad. So

FIG. 6.

far as we can tell from the sections the pad might have formed after the bud began its development, though this is very unlikely, since in such case the function would be quite limited. Indeed the pad would lose its power of functioning normally if the bud increased in size to any extent. The ectoderm of the bud is thrown into the folds or ridges, which are characteristic of the normal tentacles, and nematocysts are limited to these ridges (*cf.* figures).

[1] "The Development of *Gonionema Murbachii*," *Proc. Acad. Nat. Sci.*, Philadelphia, p. 764, 1902.

Figs. 7, 8, 9 show only the bud and the tissues immediately adjoining. The same general features already noted are also seen here. In Fig. 7 the muscular pad on each side of the base of the bud is nearer the same size than in Fig. 6. The relation of the entoderm of the bud and tentacle is shown in about the same way, but the arrangement of the bud entoderm into two rows is not so distinctly marked. In Fig. 8 this arrangement of entoderm is scarcely indicated, the cells being more or less massed together and not showing any apparent regularity. The character of the tissue of the muscular pad is shown better as are also the ectodermal ridges. The cavity at the distal end of the bud

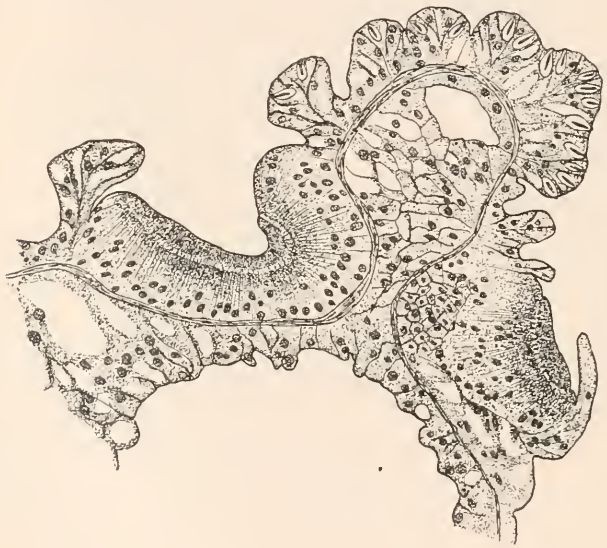


Fig. 7.

is larger than shown in the other figures. In Figs. 8 and 9 the muscular pad is shown on only one side of the base of the bud, showing that the bud is not completely surrounded by it, a feature also indicated in Figs. 3 and 5, where the pad is seen to have a notch or sinus on one side. Fig. 9 represents a section cut one side of the long axis of the bud, so that the entoderm of the bud and tentacle is not continuous.

It will have been noticed that in all the figures the bud is solid with the exception of a cavity at the distal end. Perkins (*op. cit.*, p. 785) referring to the development of the normal tentacle states

that it is at first solid, but that later "the cavity of the circular canal is drawn into it." "The entodermal cells, arranged radially about the central axis, thicken until they are forced away from the center and a tubular cavity is left." This process, he states, begins at the proximal end and the cavity is gradually "carried out along the axis of the tentacle toward the tip." In this bud there seems to be present the cavity at the distal end so

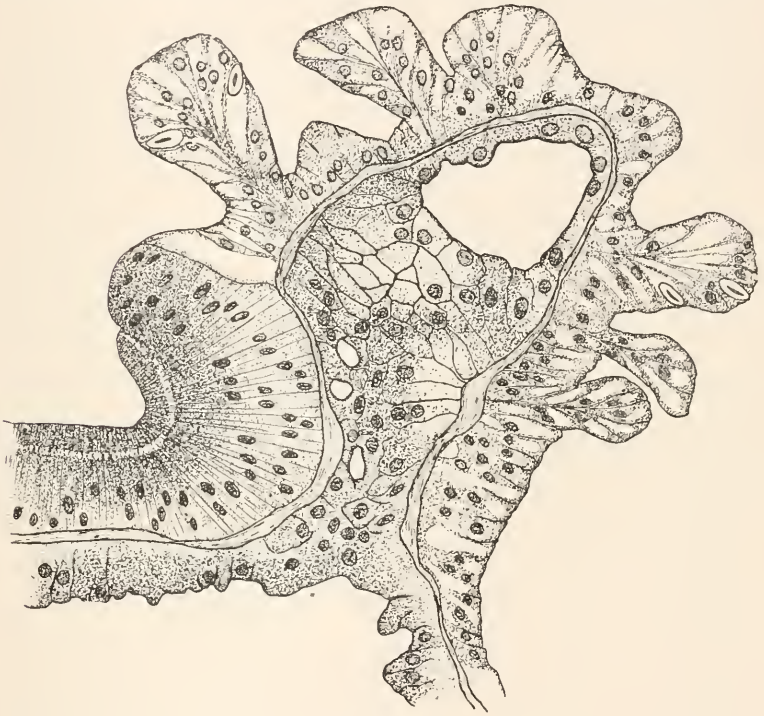


FIG. 8.

that the method just mentioned does not apply, at least not wholly. In Fig. 6 the cells are evidently arranging themselves in rows next the supporting layer, with their edges meeting near the center, suggesting a drawing away from the axis and a formation of a cavity (as Perkins suggests) connecting the cavity of the tentacle with the cavity already formed in the bud. Fig. 9, however, would seem to indicate a somewhat modified process. The cell outlines are not distinct, so that their arrangement cannot be definitely determined, but in the central part of the core are a

number of irregular cavities. This suggests the possibility of these cavities enlarging and running together, the cells at the same time taking up a regular position next the supporting layer, and thus the cavity of the bud being formed. In neither case, however, would the process necessarily begin at the proximal end. Furthermore, it is not quite certain just how the cavity at the distal end of the bud forms, or why it should form so early and not involve the proximal portion of the bud.

In regard to the early method of formation of the bud little can be suggested since it has developed beyond the initial stage. It can be said, however, that not the slightest trace of injury was found, which might be a predisposing cause. Alb. Lang[1] from

FIG. 9.

his work on budding in *Hydra*, *Eudendrium* and *Plumularia* tried to show that the bud originated from the ectoderm entirely, that the previous view that both layers were active could not be maintained ; ectoderm cells migrated through the supporting layer and formed the bud entoderm, the old entoderm being absorbed. Seeliger[2] and Braem,[3] however, working on *Hydra*, *Eudendrium*, *Plumularia*, *Obelia* and *Sertularella* claimed that Lang's results were entirely incorrect and the conclusions drawn from them

[1] *Zeitschr. f. wiss. Zool.*, Bd. 54, 1892, pp. 365–385.
[2] *Zeitschr. f. wiss. Zool.*, Bd. 58, 1894, pp. 152–188.
[3] *Biol. Centralbl.*, Bd. 14, 1894, pp. 140–161.

not warranted. They found dividing cells in the entoderm as well as in the ectoderm, a condition which Lang did not find. They found no trace of ectoderm cells migrating into the entoderm even in the earliest stages, of the two layers running into each other, of the entoderm being pushed aside and absorbed. Many others also have questioned Lang's claims and maintain that results obtained in many species of hydroids, as well as in other forms of animal life, confirm the old view. My own work on hydroids [1] confirm these results of Seeliger, Braem and others. In working on the regeneration of *Tubularia crocea*, *T. tenella*, *T. larynx*, *Eudendrium ramosum* and *Pennaria tiarella* particular attention was paid to structures which form either wholly or in part by budding. Sections made through these buds in all stages showed none of the features claimed by Lang, such as migrating entoderm cells, dissolution of the supporting layer with the accompanying disappearance of sharp contrast between the two layers, and absorption of the entoderm. Often, usually indeed, the entoderm seemed to be the layer most active in this process. Mitotic division was observed in both ectoderm and entoderm though not abundant, reasons for which are discussed in the above-mentioned paper. Amitotic division was also more or less prevalent in both layers. Perkins (*op. cit.*, p. 784) referring to the formation of the tentacle in *Gonionemus* says the three layers, ectoderm, entoderm and supporting layer "are pushed out somewhat in the growth of the tentacle, the region of greatest activity being the endodermal layer, where the core of the tentacle is formed by a rapid outgrowth of the cells of the body wall accompanied by multiplication of these same cells." He further states that there is not even an initial thickening of the ectoderm in the region where the tentacle is to appear. In regard to budding in the larval form likewise, he states that the ectoderm and entoderm cells divide, the entoderm pushes out gradually, the ectoderm growing so regularly as to cover it with a layer of constant thickness. Thus it has been conclusively proved that budding in many hydroids, as well as in the formation of the tentacle and in other buds of *Gonionemus*, involves both layers, and cells in both layers increase rapidly by division. This would entirely dis-

[1] *Arch. f. Ent-mech.*, Bd., XVII., Heft 1, 1903, pp. 64–91.

credit the universality of Lang's claims, even if they held true for a few forms.

Since this is the method of the formation of the tentacles, and of buds in other regions of *Gonionemus*, we may safely assume that probably the same process would be active in the formation of the bud from the tentacle, or at least to a great extent. Greater support is given this assumption by the fact of the presence of mitotically dividing nuclei found in the entoderm of the bud. They were not definitely determined in the ectoderm though doubtless present.

I am glad to acknowledge my indebtedness to my father, C. W. Hargitt, for permission to reproduce Fig. 2, which appeared in his paper on "Variation among Hydromedusæ," and also for allowing me to examine his collections of *Gonionemus*.

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BIOLOGICAL BULLETIN

A CRUSTACEAN-EATING ANT (LEPTOGENYS ELONGATA BUCKLEY).

WILLIAM MORTON WHEELER.

There are few more profitable fields for the comparative study of instinct than the larger genera of the social Hymenoptera. This is especially true of the larger genera of ants, such as *Camponotus*, *Formica*, *Myrmecocystus*, *Leptothorax*, *Pheidole*, *Atta* and *Crematogaster*. To these genera, each of which embraces species presenting a considerable range of ethological peculiarities while differing but little in morphological characters, we must also add *Leptogenys*, with its subgenus *Lobopelta*, a rather large tropicopolitan congeries of species belonging to the primitive Ponerine subfamily.[1]

Only a single member of this genus, *Leptogenys* (*Lobopelta*) *elongata* Buckley, is known to occur north of the Rio Grande River. It is not uncommon in the semiarid regions of Central Texas (Travis and Comal counties) and has been taken even as far north as Colorado and the District of Columbia. Frequent observations during the past three years have enabled me to confirm and extend my former account of the habits of this very interesting ant.[2]

I am now able to state positively that the peculiar apterous females, indistinguishable from the workers except for the shorter and more rounded petiolar node and the more voluminous gaster, are the only females produced in the colonies of *L. elongata*. Each colony contains only a single one of these females and no other is tolerated in the nest. Even the young virgin females leave the formicary very soon after hatching and acquiring their

[1] *Lobopelta* differs from *Leptogenys sensu stricto* in having broader mandibles which, when closed, leave little or no space between their inner borders and the anterior margin of the clypeus.

[2] "A Study of Some Texan Ponerinæ," BIOL. BULL., Vol. II., No. 1, Oct., 1900.

red adult coloration. There can, of course, be no true nuptial flight, since the females are wingless. Most conclusive evidence in regard of the nature of these females has been furnished by my former pupil, Miss Margaret Holliday,[1] who found them to possess not only well-developed ovaries but a typical receptaculum seminis. It is interesting to note that the slight morphological differences separating these females from the workers are still further diminished by Miss Holliday's discovery that the latter have as many ovarian tubules as the former and may occasionally possess a receptaculum.

That the numerous tropical species of *Leptogenys* agree with the Texan species in having very ergatoid females, is indicated, first by the fact that no winged *Leptogenys* females have been seen, though many species of the genus have been known for years, and secondly by Wroughton's observations on the Indian *Leptogenys diminuta* Smith, recorded by Forel:[2] "At my request Mr. Wroughton has excavated an enormous formicary of *L. diminuta* to a considerable depth, but has looked in vain for a female among the many thousands of workers. All he could find was a worker whose abdomen was conspicuously distended with the ovaries. This worker differed in absolutely no particular from the others, and there is nothing very extraordinary even about its abdomen. This result would seem to confirm Emery's opinion."[3]

In my paper on the Texan Ponerinæ I failed to furnish conclusive proof of the identity of the males of *L. elongata*, as up to that time I had not taken this sex in the formicaries. More recently I have repeatedly seen the males in the natural nests and have bred them from larvæ and cocoons in captivity. They are of a rich yellow color, retaining throughout life the tint exhibited by the workers and females only during their callow stages. Even when quite mature the males are seized by the workers, when-

[1] "A Study of Some Ergatogynic Ants," *Zool. Jahrb. Abth. f. Syst.*, Bd. XIX., Heft 4, 1903, pp. 295–297.

[2] "Les Formicides de l'Empire des Indes et de Ceylan," Part VII., *Journ. Bombay Nat. Hist. Soc.*, Vol. XIII., p. 312.

[3] Emery advanced the opinion that in the genus *Leptogenys* the function of the females may have been usurped by the workers. This is not strictly true, at least in *L. elongata*, since the petiole of the female is clearly different from that of the worker, as it is in the winged females of many other species of Ponerinæ.

ever the nest is disturbed, and carried away like the very slender larvæ and cocoons, with their bodies tucked away between the legs of the workers. The males leave the nests at night, like the peculiar males of *Eciton* — another genus in which the females are apterous — and are often lured into the houses by the electric lights during the late spring and early summer months, especially during the latter part of May and early June. It would be extremely interesting to learn something of the mating habits of these highly heliotactic males and wingless females. Do the males, during the breeding season, seek out and enter strange nests of their own species in order to fecundate the virgin females? This seems improbable when we stop to consider that male ants are so very stupid that they are unable to find their way back to their parental nest when once they have strayed away from it. Are the wingless females fecundated by the males of the same colony, *i. e.*, by the offspring of the same mother? This is possible but improbable, since this would be a flagrant case of inbreeding. It seems more likely that the virgin females leave the parental nest and wander about as pedestrians, till they are found and fecundated by the winged males, as in the case of the Mutillidæ. The same problems and answers seem to be suggested by the large winged males and dichthadiiform females of *Eciton* and *Dorylus*.

My former paper contained no account of the feeding habits of *L. elongata* in a state of nature. In my artificial nests the insects and their larvæ were fed on termites. I have since found that these ants, under natural conditions, feed very largely, if not exclusively on the common wood-slaters (*Oniscus* and *Armadillidium*) which abound under stones and logs in the shady places where the formicaries are excavated. I have repeatedly seen workers of *L. elongata* returning to their nests, carrying dead slaters in their mandibles. The earth surrounding the entrances to the nests is invariably white with innumerable bleaching limbs and segments of the crustaceans, showing that great numbers of these animals must be habitually destroyed by the ants. Their long, toothless mandibles resemble scissors and seem to be admirably adapted for cutting through the intersegmental membranes of their prey and exposing the

edible parts. *L. elongata* is, to my knowledge, the only ant known to feed on crustaceans as a regular diet. Other ants are either insectivorous, granivorous, mycetophagous, or collectors of the saccharine exudations or juices of insects and plants.

The little that is known concerning the habits of other species of *Leptogenys* would indicate that the North American form is peculiar in the character of its food. Wroughton has studied the habits of two of the Indian species of *Lobopelta*, *L. distinguenda* Emery and *L. chinensis* Mayr.[1] Concerning the former he writes as follows: "This species is fairly common from Poona westwards to the Ghats. The idea of a disciplined army has been fairly developed in this genus. *L. distinguenda* may sometimes, it is true, be found loafing about singly, but these individuals are probably only scouts; ordinarily, she is only met, in the early morning or late in the afternoon, travelling in an unbroken column four to six or eight abreast, straight, or rather by the easiest road, to the scene of operations. This is usually a colony of white ants whose galleries have been broken open by the hoof of a passing beast, or some similar accident. Arrived at destination, each worker seizes her termite prey, tucks it under her thorax in the orthodox ponerine fashion, and the column then returns (but marching 'at ease' and much less regularly than on the outward journey) to the nest. I have never succeeded in finding a nest; on one occasion I tracked a column for more than fifty paces, only to lose it in a patch of prickly pear. I do not think that *L. distinguenda*, any more than any other ant, ever has the inspiration to open a termite gallery for herself; on the occasion mentioned above, the column passed close to several, and even over one colony of white ants before reaching its destination; I believe, however, I saw a worker break open a piece of tunnel, into which a termite had retreated, but cannot be sure, and the practice certainly was not general. Nor are the termites followed into the galleries, partly, perhaps, because the passage is too small for a *Lobopelta*, but equally, I imagine, because such a measure would be very like 'drawing' a badger 'only more so.' Mr. Aitken tells me he has seen 'hundreds going into a hole in the ground and emerging with white ants,' but this is very different from entering a termite gallery."

[1] "Our Ants," *Journ. Bombay Nat. Hist. Soc.*, 1892, p. 56–58.

Concerning *L. chinensis* Wroughton makes the following statements: "This species is even commoner than the last. *Distinguenda* would seem to be a denizen of forests, while *Chinensis* prefers more open and inhabited country. I have only once seen *Chinensis* on the warpath, and then the objective, a large worm, in several pieces, had been reached, and the column was on its way home. The column I must say was more a mob than a disciplined army, but this may have been due to the fact that the normal irregularity of the homeward march was enhanced by the size and shape of the booty, which did not admit

Leptogenys (*Lobopelta*) *elongata* Buckley, male, female and worker.

of being carried 'according to the regulations.' On the other hand, I have often, during the early part of the rains, witnessed a migration (or was it a colonization, in no case was a female, even apterous, present?) when the discipline and regularity of the column left nothing to be desired. My experience seems to show that *Chinensis* prefers a formation in fours, at any rate when carrying her own larvæ and pupæ. Mr. Aitken has furnished me with the following most interesting note on *Chinensis*. 'There is a populous community of this ant, in a hole, in the

foundations of my house, at Goa. From the nest there is a well marked "road," crossing a broad gravel path, and then ramifying all over the tennis ground. They issue after sunset, and march along one of the main branches, or break up into parties and take different routes. When they come to a place where the termites have thrown up new earthworks, and are busy eating the dead grass underneath, they collect in dense masses, waiting for an opportunity of breaking in, which they very likely find when the termites attempt to extend their works on any side. Then the slaughter begins. Sometimes the poor termites are killed far faster than they can be carried off; and on one occasion, as late as 7 a. m., I saw the ground still heaped with slain, and an unbroken stream of ants, fifty-six yards long, carrying them away. Each ant had two or three in her jaws. If these ants cross the grounds of a community of 'harvesters' (*? Holcomyrmex*) after the latter are up in the morning, they have to flee in their turn. A *Lobopelta*, when once a worker major has laid hold of her by the leg, appears to be perfectly helpless, she can neither kill her enemy nor shake her off. Sometimes another *Lobopelta* will come to her assistance, and, after vainly trying to tear off the aggressor, will pick up her comrade and carry her and her enemy off together.' "

Apparently some of the American species of *Leptogenys* also prey on termites. The nest of a single colony of *L. Wheeleri* Forel which I observed at Cuernavaca, Mexico, was almost embedded in a *Eutermes* nest, and I have no doubt that the ants were in the habit of using their neighbors as a convenient larder.

The only other *Leptogenys* of which I find the habits recorded is *Lobopelta diminuta* Smith var. *bismarckensis* Forel from the Bismarck Archipelago. Dahl[1] compares this species with the amazon ant (*Polyergus rufescens* of Europe and North America). on account of the sickle-shaped, toothless mandibles. He says: "Although I have drawn a comparison between *Leptogenys bismarckensis* and *Polyergus rufescens*, I must clearly emphasize the fact that I never found the nest of the former species and

[1] "Das Leben der Ameisen im Bismarck-Archipel, nach eigenen Beobachtungen vergleichend dargestellt," Berlin, R. Friedländer und Sohn, 1901, p. 52.

could not, therefore, observe whether the work of the colony is carried on by slaves. What firmly convinced me, nevertheless, that this species is a slave-holding ant, was the following: The mandibles are long and sickle-shaped, almost as much so as in our German slave-holders, and little adapted for working, as they have no masticatory border. They could of course, be of use in killing termites, as Forel supposes to be the case in this genus, but the place were I found the species under consideration was far removed from all termite nests. It was on the sea-shore between blocks of coral, where I saw about fifty individuals in a troop, as it were, on the march. It was evident that the troop had left the nest for the purpose of perpetrating a robbery in common. That this robbery, after what has been said, was for the purpose of obtaining slaves, seems probable." I venture to maintain that Dahl is mistaken in supposing that *L. bismarckensis* is a dulotic ant, as a perusal of the above quoted passages from Wroughton's work will suffice to show.

It is also evident from the observations of Wroughton and Aitken that the Indian species of *Leptogenys* differ widely in habit from *L. elongata*. The colonies of the latter species are very small, rarely containing more than a hundred individuals, whereas the colonies of the Indian species appear to contain thousands of workers. Moreover, the workers of *L. elongata* leave the nest singly and hunt about timidly for their phlegmatic and defenceless prey, whereas the Indian species hunt in well organized files somewhat after the manner of the driver ants and ants of visitation (*Dorylus* and *Eciton*). And such diversity of instinct is exhibited not only in the same genus but within the confines of the same subgenus (*Lobopelta*).

In conclusion I give the synonymy and a description of all three phases of the North American *Leptogenys*.

LEPTOGENYS (LOBOPELTA) ELONGATA (Buckley) Wheeler.

Ponera elongata Buckley, Proc. Ent. Soc. Phila., Vol. VI., 1866–67, p. 172, ♀.

? Ponera texana Buckley, ibid., p. 170, ♀.

Lobopelta septentrionalis Mayr., Verhandl. k. k. zool. bot. Ges. Wien, Bd 36, 1886, pp. 438, 439, ♀.

Leptogenys septentrionalis Emery, Zool. Jahrb. Abth. f. Syst., Bd. 8, 1894, p. 268, ♀.

Leptogenys (*Lobopelta*) *elongata* (Buckley) Wheeler, Biol. Bull., Vol. II., No. 1, Oct., 1900, p. 2, 7, Fig. 4. ☿ ♀ ♂ ; Trans. Tex. Acad. Sci., Vol. IV., Pt. II., No. 2, 1902, p. 9

Worker. — Length 5–6.5 mm.

Head slender, excluding the mandibles, longer than broad, somewhat broader in front than behind. Eyes moderate, flattened, situated a little in front of the middle of the sides of the head. Mandibles slender, nearly two thirds as long as the head, gradually increasing in breadth as far as their apical third, forming a sharp, toothless blade and thence narrowing more suddenly to the acute, curved tip. Clypeus prolonged forward in the middle to a rather acute point and with a prominent median keel ; lateral emarginations distinct but not very deep. Frontal furrow extending back a little beyond a line connecting the posterior orbits of the two eyes. Antennæ slender ; scapes extending about one third their length beyond the posterior corner of the head. First and third funicular joints subequal, decidedly shorter than the second joint ; joints 4–11 subequal, shorter than joints 1 and 3. Thorax elongate, its dorsal surface horizontal, meso- and epinotal regions laterally compressed ; depression behind the small mesonotum short but rather deep. Basal surface of epinotum twice as long as the sloping declivity, which is somewhat flattened and transversely marginate below and behind. Petiole in profile as high as the thorax, somewhat higher behind than in front and nearly as long as high ; anterior, dorsal and posterior surfaces flattened ; the dorsal and posterior meeting at a somewhat sharper angle than the anterior and dorsal surfaces. There is a distinct tooth at the anterior, ventral border of the petiole. Seen from above this segment is pyriform, twice as broad behind as in front, with flattened sides and rounded dorsal surface. Gaster slender, somewhat deeper than the petiole, distinctly constricted between the first and second segments. Sting prominent. Legs long and slender ; claws with long pectination.

Mandibles smooth and shining, the former with a few scattered punctures and faint traces of striation ; the latter thin and submembranaceous along its anterior border and obliquely rugose on the sides. Head, thorax and petiole subopaque, rather densely and uniformly covered with shallow punctures. Region between the eyes and clypeus longitudinally rugose. Gaster shining, very sparsely and finely punctate.

Body and appendages clothed with delicate, grayish yellow pubescence ; the head, thorax and abdomen also with sparse, grayish yellow hairs, which are long and projecting on the clypeus and terminal segments of the gaster.

Deep red ; edges of mandibles and thoracic sutures somewhat blackened. Antennæ and legs a little paler than the trunk ; tip of gaster, sting, anterior border of the clypeus and the spines of the tibiæ, yellow.

Female. — Length 6.5–8 mm.

Apterous and decidedly ergatoid in form, indistinguishable from the

worker in the head and thorax, even lacking ocelli and with eyes no larger than those of the worker. The petiole is proportionally shorter and higher, so that when seen from above, it is little if any longer than broad behind, shaped like an eqilateral triangle with rounded angles. In some females the upper surface of the segment in profile is flattened like that of the worker, but in most cases it is more convex and in this respect somewhat like the petiole of the male. Gaster conspicuously larger, both broader and higher than that of the worker. Sting like that of the worker.

Male. — Length 5.5–6.5 mm.

Head, including the very prominent, somewhat reniform eyes, distinctly broader than long ; cheeks and postocular regions very short, ocelli large and protruding. Mandibles small, hardly meeting with their tips, slightly geniculate, broadest at the base and suddenly tapering to a slender point. Clypeus less produced, less pointed in front and with a blunter keel than in the worker. Antennæ long and filiform, 13-jointed ; joints 3–13 long and cylindrical, subequal ; scape very short, hardly a third as long as the third and succeeding joints ; second joint very small, somewhat narrower than the scape and hardly longer than broad. Thorax rather robust, with prominent Mayrian furrows on the mesonotum ; epinotum long and sloping. Petiole small, hardly half as high as the first gastric segment, about as high as long, in profile rounded above, with sloping anterior and posterior surfaces ; seen from above it is oblong, a little longer than broad, hardly wider behind than in front. Gaster with well developed constriction between the first and second segments and large, cultriform, exserted genital appendages, which are fully half as long as the remainder of the gaster. Legs long and slender.

Smooth and shining, except the antennæ, which are opaque. Surface of thorax indistinctly and finely punctate.

Body covered with yellowish hairs, which are long and prominent on the gaster, shorter on the head and thorax, and still shorter and denser, and more like pubescence, on the appendages.

Body and legs light yellow throughout. First and second antennal joints yellow, remaining joints brown. Wings grayish hyaline with brownish veins and stigma.

Type Locality. — Austin, Texas.

Other Localities. — District of Columbia (Pergande, Mayr) ; Colorado (Cresson, Emery) ; New Braunfels and Belton, Texas (Wheeler).

THE MARCHING OF THE LARVA OF THE MAIA MOTH, HEMILEUCA MAIA.

WM. S. MARSHALL.

In the autumn of 1901 while collecting along the marshy shore of Lake Wingra, near Madison, I noticed many specimens of the maia- or buck-moth, *Hemileuca maia*, flying low over the marsh. Both males and females were present, and many of the latter, having settled, were laying their eggs on the grass. These were placed in a somewhat irregular set of spirals closely packed together so that when they hardened, the grass could often be pulled away leaving the eggs stuck together and forming a short tube. The process of oviposition and the arrangement of the eggs has been described by Riley[1] and copied by Packard.[2]

Without having any definite plans in view I collected a great many of the eggs most of which I put in a cold place, but a few I left in an open bottle in my room. One morning I noticed on the neck of this bottle a black mass which was found to be a group of young caterpillars; they had evidently hatched but a short time before. Later in the morning I again looked at the eggs and found that more had hatched; all in the first bunch, having in the meantime left the bottle, were marching in a line on the table. Again, later in the day other groups were seen, and in nearly every instance, each group had formed a line marching in a regular procession and following the leader whichever way he turned. I placed some large sheets of paper on the table; upon these the different groups were soon marching, and could be much more easily seen than when upon the darker table. I now, with a pencil, knocked the leader away from one line and was surprised to see the next in the line, now the leader, stop when he reached the place occupied by the first leader prior to his removal. Here he stopped and raising himself upon his prolegs moved the anterior part of his body to and fro as if he

[1] Riley, C. V., "Fifth Missouri Report," p. 128.

[2] Packard, A. S., "Insects Injurious to Forest and Shade Trees," Washington, 1890.

were trying to scent the leader. He soon discontinued this and resumed his natural position again, appearing, however, for some time, very restless. While this had been going on, the rest of the caterpillars had crowded up to the front one; they appeared for some time very restless, but finally settled in a close bunch, in which position they all remained: one here and one there would often become restless for a few minutes, but end by settling again in its former position in the bunch. I now marked the original leader (he had been kept away from the others all of this time) by putting a little white paint on his back and then picking him up on a small piece of paper dropped him back at the edge of the group. The leader, in the meantime, had been walking around evidently seeking the other caterpillars, and when he returned to the bunch, began to walk restlessly around near its edge. In a few minutes he started off away from the others and these began to follow him, moving in a regular procession.

Different masses of the eggs were now brought into my room, a few each week, and when the caterpillars hatched, a few experiments were carried on to see if the removal of the leader always affected the followers in the same way, and if a new caterpillar would not assume the leadership and be followed by the rest. The results I have thought it best to write out briefly and not to arrange them in a tabulated form. The first set contains those in which the old leader, upon being returned to the bunch, resumed command, and the second lot, those experiments in which a new caterpillar became the leader. There then follow a few experiments differing from those contained in the first two lots. When the number of caterpillars forming the line was counted, this is given; but in some cases, however, the number which formed the line was not noted.

In nearly every instance the removal of the leader brought about at first the same result. When I removed him I would draw a line on the paper marking a place where his head was before removal. When the second caterpillar in the line reached this point, he always stopped, rarely crossing the line, and when the bunch was formed, it was always back of the line.

1. Sixty-four in line. 8:15, leader removed; 8:30, all bunched; 8:31, leader returned; 8:35, all restless; 9:00, leader started, and at 9:08, all were in line and moving.

2. Six in line. 8:45, leader removed, others soon bunched; 8:51, leader returned; 8:57, old leader started; others following.

3. 8:47, leader removed; 8:50, all bunched; 8:51, leader returned; he passed to back of bunch and started away, others following; 8:43, all in line.

4. Ten in line. 8:31, leader removed and all bunched one inch back of line; 8:36, leader returned; 8:55, leader starts and others follow.

5. 8:57, leader removed and put back at once, seventh in the line; all bunched; 4:06, P. M., leader started, others following.

6. 8:16, leader removed; 8:26, leader returned; 8:31, leader started, others following.

7. 8:21, leader removed; a caterpillar, the next in line, goes on half an inch and here they all bunch; 8:30, leader returned; 8:42, leader starts and others follow.

8. 2:32, leader removed; 2:47, leader returned to bunch; 3:55, leader started, others following.

9. One hundred and fifty in line. 10:22, leader removed; 10:40, bunched and leader returned; 11:25, leader started and others followed.

The following are experiments where a new leader started:

1. Forty in line. Leader removed; 9:10, bunched; 9:11, a caterpillar at back of bunch starts and others follow.

2. Twenty-one in line. 9:01, leader removed; 9:08, bunched; 9:10, one near front starts; 9:21, all in line following new leader.

3. Eighteen in line. 8:50, leader removed; in a minute the next caterpillar turned and started; 8:55, all in line and following new leader.

4. Thirty-six in line. 8:42, leader removed and the next caterpillar assumes leadership; 8:47, new leader removed and original one put back in middle of line; all bunch; and at 4:08, P. M., were still quiet.

5. Eighteen in line. 8:30, leader removed; 8:37, bunched; one went a short distance over the line but returned; leader returned; 8:46, new leader started and others follow.

6. Eleven in line. 8:38, leader removed; 8:41, next caterpillar returned along the line two inches and then came back; others bunched; 8:45, start with new leader.

7. Ten in line. 10:34, leader removed; line kept on; 10:39, second leader removed; march continued; 10:40, third leader removed; 10:42, bunched; 10:48, started with the original leader, who had been returned, at head.

8. 8:13, leader removed; 8:22, leader returned; 8:56, new leader started, others following.

9. 2:56, leader removed; 3:05, leader returned; 4:05, new leader started and others follow.

10. Forty-six in line. 9:24, leader removed; 9:33, all bunched; leader returned; 10:05, new leader starts and others follow.

11. One-hundred and twenty in line. 8:41, leader removed; when the entire line had bunched the leader was returned; 11:45, new leader starts and others follow.

12. One-hundred and fifty in line. 8:43, leader removed; 9:25, mostly bunched and leader returned; 10:10, old leader started, rest following; they went more than an inch and then returned to bunch again; 2:30, P. M., a new leader starts and others follow.

The following experiments were made by removing the leaders of two lines and then returning them each to the other's bunch:

A. Six in line. 2:02, leader removed; 2:06, bunched; leader of *A'* placed near head; 2:08, leader of *A'* starts and others follow.

A'. Thirty-two in line. 2:02, leader removed; 2:07, leader of *A* placed in bunch; 2:11, one, not the leader, started but returned; 2:35, leader of *A* starts and others follow.

B. Thirty-two in line. 1:56, leader removed, when others bunched leader of *B'* placed with them; 2:18, leader from *B'* starts and others follow.

B'. Thirty-five in line. 1:56 leader removed and leader of *B* placed in bunch; 2:03, leader of *B* starts and others follow.

C. 1.22, leader removed; 1:27, leader of *C'* placed in bunch; 3.20, line starts with entirely new leader.

C'. 1:22, leader removed; 1:27, leader of *C* placed in bunch. Called away and unable to follow this to end.

The following experiments I give separate from the others:

1. 8:24, leader removed; 8:33, leader returned and at once started out, the other caterpillars remaining in bunch; 8:38,

leader returned again ; 8:42, he starts out again but none follow ; in two minutes he returns to the bunch himself and starts a third time, this time some follow but soon return to bunch and leader goes off by himself.

2. Eighteen in line. 8:32, leader removed ; 8:38, bunched ; leader returned and they remained in bunch all morning.

3. Thirteen in line. Same thing happened as in number two.

In one lot, thirty-six in line, the leader had been marked and the line allowed to go on. The leader reached the last caterpillar in the line so that a circle was formed. They all kept moving, the leader finally reaching the place where he was when the circle was first formed ; he then went half way around the circle and started off in another direction the others following.

From the above, it will be seen that the removal of the leader affects the whole line, but that he is not necessary for the further progression of the caterpillars. I have been unable to find references to the procession-caterpillar, but notice that the caterpillars of *Saturnia io*[1] march when young the same as the Maia-moth.

Dubois[2] notes that the procession-caterpillar spins a thread which the others in the line follow ; the young larvæ of *Hemileuca* do the same, the thread being seen with a hand lens back of the line. In nearly all of the long lines, which the larvæ form, there is very apt to be at least one break where there is an inch or more between the nearest caterpillars, or such a break can be made by stopping one of the larvæ until the preceding ones have gone ahead for some distance : at such a place the thread also can be seen. When a break occurs, it does not in any way affect the movements of the line, the caterpillars following along the regular path.

Wishing to see how much the caterpillars depended upon this thread to enable them to follow in the exact path of the leader, I removed the thread a number of times when the distance between two neighboring caterpillars was great enough, and found that the course was not in the least altered. The caterpillars, upon reaching the end of the broken thread, generally kept straight on as if nothing had been done, failing to show a dependence

[1] Dickerson, Mary C., "Moths and Butterflies," 1901.

[2] Dubois, *Ann. Soc. Linn. Lyon.*, XLXI., 1900, p. 125.

upon the thread alone in following the path of those ahead. I next removed the thread, and then dipping a finger in water, rubbed it rapidly a number of times across the path and then wiped the place dry. When the first caterpillar reached this spot, he halted, and for three minutes remained at the same place, raising the anterior part of the body in the air acting the same as if the leader had been removed. At the end of this time he started forward following, as near as I could judge, the original path.

The following few experiments should have been made with the food plant of the caterpillar, but this being unobtainable at the time of the year when the caterpillars were hatching in my room, the leaf of the geranium, *Pelargonium*, which was easily obtained and possessed quite an odor, was used.

1. A small piece of the leaf was placed 5 mm. away from a small group which had been quiet for two or three hours; the caterpillars became at once restless and in two minutes, three had moved over and touched the leaf.

2. A small piece of the leaf was placed 5 mm. away from the leading caterpillars in a line; they became at once restless and "broke rank"; in four minutes two (not the leader) had reached the leaf.

3. A piece of leaf was placed 15 mm. from a group, nothing happened; the leaf was moved to 10 mm. and left for ten minutes, nothing occured; moved to distance of 5 mm. from caterpillars and all still remained quiet. I now moved the leaf to 3 mm. away, one immediately came out, touched the leaf and returned to its original position, in thirty seconds another came out, touched the leaf and returned.

4. A piece of leaf was placed 5 mm. from group, two came out, touched it and returned.

Zoölogical Laboratory, University of Wisconsin,
Madison, February, 1904.

FORM-REGULATION IN CERIANTHUS, IV.

The Rôle of Water-pressure in Regeneration.

C. M. CHILD.

Introductory.

The regeneration of the marginal tentacles in cylindrical pieces was described in the first paper of this series (Child, '03*a*).

The tentacles do not arise from the new tissue closing the end, nor from the cut surface, but from the old body-wall itself, which first becomes thinner and loses its muscular layer in the region where the tentacles are to appear and then gives rise to small buds, corresponding in number and position to the intermesenterial chambers.

The fact that the new marginal tentacles arise some distance away from the cut surface by a local transformation of the already differentiated body-wall is of considerable importance. There must be some adequate ground for the localization of this peculiar process of transformation in a region of the body-wall which apparently differed in no way from adjacent parts before the cut was made.

Very early in my study of regeneration in *Cerianthus* it became evident that the rapidity of regeneration was more or less closely connected with the degree of distension of the piece. My observations along this line were made chiefly upon *C. solitarius*. In cases where closure of the ends and consequently distension by water was possible regeneration was much more rapid than in pieces where communication between the enteron and the exterior — other then the mouth and the aboral pore — existed. Observation of this apparent relation between water-pressure and regeneration led to experiment and it was soon possible to demonstrate that a very close relation between water-pressure in the enteron and regeneration existed. I am aware, of course, that Loeb ('91, '02) regards certain phases of this relation in *Cerianthus* as osmotic in nature. The results of his experiments and his interpretation will be discussed at another time: it need only be

said here that Loeb failed to understand the real conditions, otherwise he would scarcely have attempted to apply the osmotic hypothesis to this case.

The evidence bearing upon the relation between regeneration and internal water-pressure is varied in character. In the remaining portion of the present paper and in following papers the various lines of evidence will be discussed. First, however, it is necessary to call attention to certain features of the water-pressure and circulation in the enteron of normal animals. Except where statement to the contrary is made all observations and experiments were made upon *C. solitarius*.

Water-pressure and Circulation in the Enteron.

Under ordinary conditions both the body-wall of *Cerianthus* and the tentacles are subjected to a considerable degree of tension in consequence of internal water-pressure, the enteric cavity being distended with water. When contraction occurs this water issues through the aboral pore, at least in large part, though some passes out through the stomodæum. After loss of water from the enteron the body and tentacles are shorter and smaller, and, if the loss was great, are more or less completely collapsed. This condition continues until the enteron again begins to fill with water. As it fills, the body gradually resumes the original form exactly as does a rubber balloon when inflated with air. This distension of body and tentacles by water under pressure is an indispensable condition of the characteristic form of body and tentacles. The animal is incapable of extending to its full length or attaining its full diameter in any other way than through the medium of the internal water-pressure. Extension of the tentacles and the turgid condition often designated as erection is just as completely the result of the enteric water-pressure. Turgor of individual cells has absolutely nothing to do with the condition, as is evident to all who have examined with any care *Cerianthus* or other actinians. Loeb's belief ('91) that osmosis plays a part here is wholly without foundation, except so far as water may diffuse through the body-wall into the enteron.

We may regard *Cerianthus* as a sac filled with water under pressure. Under certain conditions the walls of the sac may de-

crease in size either locally or at all points, the result being in the first case increase of pressure and either stretching of other parts of the sac or loss of water through the pore or mouth, and in the second case always loss of water, commonly through the pore, and reduction in size of the whole body. While this sac has the power of contracting and forcing water out through certain openings, it has not the power of expanding actively and so drawing water in.

Since water under pressure is normally present in the enteron, and since after collapse due to contraction the pressure is soon reëstablished, means must exist by which water can be forced into the enteron against pressure. In my first paper (Child '03*a*) mention was made of the fact that regenerating pieces closed at both ends by their membranes of new tissue become distended with water in the course of a few days. It was suggested that in such cases water diffuses through the body-wall in consequence of the accumulation within the enteron of certain soluble products of metabolism. It is also possible that water may be secreted into the enteron together with these substances. To what degree the distension in normal animals is caused in this manner cannot be definitely ascertained, but it is certain that the rapid accumulation of water in the enteron of the normal animal after this has lost all or nearly all of its contents in consequence of violent contraction cannot be due to diffusion or secretion. In such cases the animal frequently regains its usual degree of distension within fifteen or twenty minutes, whereas distension by diffusion in regenerating pieces requires several days.

The few observations which have been made upon the siphonoglyphes in actinians indicate that the function of these structures is the production of currents through the stomodæum. Hickson ('83) found that in certain Alcyonaria, which possesses only one siphonoglyphe, the current was inward or downward along the siphonoglyphe and outward on the other portions of the stomodæal surface. It is probable further, according to Hickson, that the inward or downward current is continued along the free edges of the mesenteries for a greater or less distance below the siphonoglyphe, while on other mesenterial margins the cilia beat in the reverse direction, like those of the general stomodæal surface outside the siphonoglyphe.

According to this view there is an inward and an outward current in the stomodæum and for a greater or less distance aboral to it in the central region of the enteron between the margins of the mesenteries. According to my own observations conditions are very similar in *Cerianthus*. Only one siphonoglyphe is present (Fig. 1) and along this the current is directed inward, though whether the cilia may ever reverse this movement has not determined. The fact that small particles placed on the disc, if not ingested by muscular action, are gradually carried in a peripheral direction indicates that here the cilia beat so as to cause an outward current. The direction of the current on the lower parts of the œsophagus outside of the siphonoglyphe has not been determined, but is probably the same as upon the disc and upper portions. In Fig. 2 the opposing stomodæal currents are indicated by arrows, the inward current along the siphonoglyphe by the longer arrow on the left and the outer currents by two shorter arrows on the right.

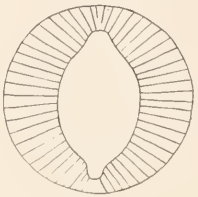

FIG. 1.

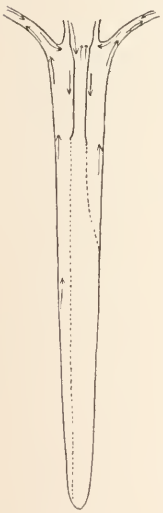

FIG. 2.

The natural conclusion is that the accumulation of water in the enteron is in some way the resultant of the stomodæal currents, but the manner in which this result is attained requires consideration. When the enteron is distended with water under pressure the walls of the œsophagus must be brought into contact since pressure is exerted upon them from all sides by the water in the enteron. The radiating grooves corresponding to the lines of attachment of the mesenteries, which are visible upon the disc of *Cerianthus*, extend the whole length of the stomodæum and probably it is along these that the chief outward currents pass. As the walls of the œsophagus are pressed more more closely together these grooves are more and more nearly obliterated. Probably when internal pressure is high the outward currents are very slight or absent. The siphonoglyphe, however, is a relatively powerful organ, which produces an in-

ward current. This groove is probably always open whether the body is distended or not. According to the laws of fluid pressure the force of the inward current need be only slight since the pressure it exerts is transferred to every unit of surface and in all directions in the enteron, *i. e.*, the principle by which enteric pressure is maintained is that of the hydraulic press ; a slight pressure exerted over a very small surface is transferred to a large body of confined fluid and so multiplied many times.

If these suggestions are correct the passage of water in and out of the body and the process of distension and collapse are accomplished somewhat as follows : When the body is contracted it is probable that the œsophagus is more or less widely open ; observation of living animals or sections of contracted specimens (Fig. 1) support this view. This condition of the œsophagus is probably brought about by contraction of the mesenteries which possess a slightly developed transverse musculature. In the contracted state then there is free passage of water in and out. When the muscles relax the œsophageal walls are again brought into more or less close contact, this result being, perhaps, brought about in part by the elasticity of the œsophageal walls or by muscular contraction, and in part by the circulatory currents in the intermesenterial chambers on all sides of the œsophagus (see Figs. 1 and 2). The approximation of the œsophageal walls decreases the outward currents, while the inward current continues, since the siphonoglyphe remains open. Thus, more water enters than passes out, and the result is of course gradual distension of the body. As the internal pressure increases the walls of the œsophagus are more and more closely appressed and thus the outward currents are decreased still further while the inward current continues undiminished. The increase in pressure and distension will continue until the motive power exerted by the cilia of the siphonoglyphe is just sufficient to balance the internal pressure and prevent an outward movement of water, and to replace any loss through outward currents, if such still continue, as perhaps they do to a slight extent. So long as the various conditions remain unchanged distension continues.

Let us now suppose that a sudden stimulus leads to contraction of the body-muscles. In the first stages of this contraction

the internal pressure must be greatly increased and probably the œsophageal walls are more closely appressed than before, since the muscles which separate them are weak. At this stage then little or no water escapes through the œsophagus. When the pressure reaches a certain point the aboral pore is forced open and rapid ejection of water occurs with considerable force. Thus the internal pressure is relieved, and now, as further contraction occurs, separation of the œsophageal walls takes place, and the remaining water passes out of the œsophagus, sometimes carrying with it mesenterial filaments in case of violent contraction. The point to which I desire to call especial attention is that when contraction begins the internal pressure closes the œsophagus all the more tightly, and it is not until this pressure is relieved by escape of water through the aboral pore that opening of the œsophagus can take place. Though few observations have been made on other species of actinians, I am inclined to believe that distension and collapse may be accomplished in much the same manner in other members of the group, the cinclides or other openings taking the place of the aboral pore. It is probable, however, that in many forms the muscles which separate the œsophageal walls are more powerful than in *Cerianthus* and so are able to bring about separation in spite of the internal pressure. It is perhaps needless to state that the ejection of mesenterial filaments through the cinclides or mouth is purely passive, the filaments being merely carried out with the water.

One other point may be mentioned in this connection: if the preceding observations and suggestions are correct it follows that the form of the œsophagus usually observed in transverse sections, viz., an oval with grooves at one or both ends (Fig. 1) is not what might be called the natural form, but a form resulting from contraction. Practically all fixed specimens of actinians are more or less contracted. It is extremely probable that when the animal is distended in the normal manner the œsophageal walls are always closely appressed except in the region of the siphonoglyphe. Dilation occurs of course in the taking of food, but it probably does not extend over the whole length of the œsophagus at one time and so does not cause any great loss of water. That the œsophagus is widely open in contracted

actinozoa is very evident from the frequent forcing out through it of mesenterial filaments; that it cannot be widely open during distension of the body by water is equally evident. Doubtless many differences in detail occur in different members of the group, but it is probable that the suggestions given here for *Cerianthus* will apply more or less closely to a large number of forms. The presence of two siphonoglyphes must condition certain modifications in these processes. The statement is made in several text-books that the siphonoglyphes serve to keep the water in circulation when the animal is contracted. According to the view given above, these organs serve rather to permit circulation when the body is distended.

In addition to the inward and outward currents in the stomodæal region the water in the intermesenterial chambers and in the tentacles connected with them is in constant circulation, impelled by the movement of cilia. I have not been able to determine the details of this circulation in *Cerianthus*, but have observed it in several transparent members of the group. In general it may be said that the current passes orally along the body-wall, peripherally along the aboral face of the tentacle, back again along its oral face, centrally beneath the disc, probably into and out of the labial tentacles, to the stomodæum, and aborally along the stomodæum. Whether cilia on the lateral surfaces of the mesenteries aid in forcing the water aborally I do not know. In the forms observed, at any rate, the aboral current continued from the aboral end of the stomodæum to the aboral end of the body. In all of the intermesenterial chambers are these circulatory currents. In *Cerianthus* itself they are difficult to demonstrate with certainty, but there is little doubt that the current in the oral direction along the body-wall exists, and this being present, we may confidently assert that the return current is also present. These currents in the intermesenterial chambers and tentacles may be designated for convenience as the circulatory currents in distinction from the inward and outward currents of the stomodæal region. They are indicated diagrammatically by arrows in Fig. 2.

How far the circulation of water in the central regions of the body is complicated by the complex arrangement of the mesen-

terial margins and mesenterial filaments cannot be determined. Moreover, these are merely matters of detail and do not affect the general outlines of the process. Since the position of the organs is subject to considerable variation the direction of local currents produced by their cilia must vary likewise.

This discussion of the internal circulation and pressure has been somewhat detailed, since I believe that the general internal pressure and perhaps also local pressure resulting from the impact of currents against the body-wall are important factors in form-regulation and without doubt also in development.

Internal Pressure, Growth and Form-regulation — A Preliminary Survey.

It is necessary before proceeding to the account of my experiments to indicate briefly how growth and form-regulation may be affected by the internal water-pressure. It would be difficult otherwise in many cases to show the bearing of the experiments without extended explanation.

It is conceivable that the presence of water in the enteron may affect the body-wall in two ways: first, by general pressure, the same in all parts of the body, which subjects the body-wall to tension; second, by local pressure resulting from the impact of definitely directed currents upon some portion of the body-wall which interrupts their course, thus subjecting that part of the body-wall to a localized tension in addition to the general tension.

The experiments to be described demonstrate clearly that regulation in *Cerianthus* is dependent in certain important respects upon the tension resulting from internal water-pressure. The tissues react to this tension by growth. In the absence of the tension the typical form of the animal does not appear. Up to this point the evidence afforded by the experiments can scarcely be doubted or refuted.

An essential feature in form-regulation in *Cerianthus*, as well as in other forms, is localized growth which is especially noticeable in the formation of new tentacles.

The problem of the cause of localized growth is one of the greatest importance in morphogenesis. According to one view the basis of morphological form is inherent in the organism,

either in the structure of the protoplasm or as a governing principle distinct from physical and chemical factors to which the latter are subordinated. On the other hand, it is possible, at least theoretically, to regard organic form as the resultant of the complex of physical and chemical conditions internal and external which affect the protoplasm. According to this view, organic form is not strictly speaking inherent in the living substance; but results indirectly from its activities and its environment.

The experiments to be described demonstrate, I believe, that in the absence of internal water-pressure the living substance of *Cerianthus* is absolutely incapable of producing the typical form of this animal. It is not merely that the parts are formed and remain collapsed in the absence of water-pressure; either they are not formed or their form is atypical. The appearance of the tentacles is delayed or is inhibited; the growth of the disc and the œsophagus does not occur when the internal pressure is reduced below a certain point. Thus it is possible in this case to demonstrate a general relation between internal water-pressure and growth.

The question as to whether any relation between localized growth and localized internal pressure exists must not be confused with the preceding question as to a general relation between the two phenomena. It is possible for instance to suppose that the region where new tentacles shall appear is determined in some unknown manner, but that the internal pressure is a factor in causing their growth after their position has been determined. On the other hand, it is possible to conceive that the position of these structures is determined by local pressure due to currents in the enteron (the "circulatory currents"). It cannot be claimed for my experiments thus far that they decide which of these two possibilities is correct. Certain of the data, however, appear to me to indicate that the local pressure due to currents may play a certain rôle in determining the position of the marginal tentacles. Concerning the labial tentacles there is as yet no definite evidence and this fact must constitute a weak point in the evidence, at least until further experiments can be performed.

Time need be taken here only for a brief consideration of the possibility of a direct relation between local internal pressure and

the determination of localized structures at certain points. In each intermesenterial chamber the circulatory current passing orally along the inner surface of the body-wall (Fig. 2) must strike against any part of the wall which is folded or rolled over in such a manner as to form an obstacle in its course. When a transverse cut in the body-wall of *Cerianthus* is made the cut edges roll inward in such a manner that the current passing orally in the chambers must strike the inner surface of the inrolled portion. In this condition that portion of each intermesenterial chamber just beneath the inrolled cut margin forms a blind sac into which water is continually being forced by the current. The cilia which in the normal animal provide for a return current have been in large part removed from this region. Local pressure upon the body-wall in this region must result. Now the marginal tentacles always make their appearance in exactly this region, a region which previously formed a part of the lateral body-wall, and which, since tentacle-regeneration is possible at any level except the extreme aboral region, cannot have possessed any special qualifications for tentacle formation. The question is, what causes tentacles to appear in this particular region of a piece, no matter what part of the body the original piece represents. Doubtless very few will regard the assumption of an "entelechy" (Driesch) or of "dominants" (Reinke) as an answer to this and similar questions, although it may possess the merit of many other unwarranted assumptions, viz., that of being an easy way out of difficulty. If we call in "heredity" to answer the question we still have no real answer, though it is evident that tentacles of characteristic form and structure would not appear if *Cerianthus* were not concerned. Are we not justified in seeking for definite, intelligible causes or conditions of one kind or another for phenomena of this nature? Much work of recent years has given an affirmative answer to this question. In many cases undoubtedly these causes or conditions are internal, *i. e.*, in the protoplasm, but it is by no means impossible or improbable that they are external in many other cases.

Certain of my experimental results seem to me not only to admit the possibility of local pressure as a determinative factor

in morphogenesis but to afford little basis for other interpretations. I desire to give the data in descriptive form, but shall endeavor to point out their bearing in connection with this hypothesis. It should be borne in mind, however, that while the existence of a general relation between internal water-pressure and growth is demonstrated, my data upon the other problem are insufficient in my own opinion to establish a conclusion without doubt. At present, though the evidence in regard to the marginal tentacles is very strong, the impossibility of giving any special evidence concerning the labial tentacles constitutes a serious defect. The regeneration of labial tentacles is certainly delayed or inhibited by reduced water-pressure, but I have not been able thus far to demonstrate that localized pressure upon the body-wall occurs in the region where they appear. This failure is due at least in part to the fact that my attention was concentrated chiefly on the marginal tentacles during my experiments. I hope that a future opportunity for renewed experimentation may render it possible to attain more definite conclusions on this point. Attention may be called to the possibility that the two sets of tentacles possessing different functions and appearing as they do at different times and under different conditions may perhaps be determined by very different factors. General similarity of form and structure is not necessarily indicative of similar conditions of origin. Even if it should be demonstrated that the marginal tentacles arise in response to the stimulus of localized pressure it would by no means necessarily follow that the labial tentacles are similarly produced.

The Closure and Distension of Pieces.

The closure by new tissue of the two ends of cylindrical pieces has been described in the first paper of this series (Child, '03*a*). The closure consists essentially in typical cases of the connection of all parts of the inrolled cut surface of the body-wall by a thin, delicate membrane of new tissue. The result of closure in this manner of both ends of the piece is the interruption of direct communication between the enteric cavity and the exterior. Nevertheless, water rapidly accummulates within the enteron, probably in consequence of diffusion or secretion, causing marked

distension of the piece. However the fluid may enter, the distension occurs in every case, and in three or four days after section (in summer) the pieces are subjected to a considerable degree of internal pressure.

The pressure of the water being exerted in all directions tends to stretch the body-wall. The thin delicate new tissue at the ends is the weakest part of the body-wall and therefore most effected by the stretching. After closure of the ends by new tissue and before distension has occurred, the new tissue is either invisible or scarcely visible from the exterior. The inrolled edges are closely approximated and all that can be seen of the new tissue in any case is the part filling in the spaces between folds and wrinkles of the cut surface. Moreover, if the inrolled margins of the body-wall be spread apart even with the greatest care at this stage the new tissue does not stretch to any great extent but is ruptured even by slight tension. Under these conditions the cut

FIG. 3.

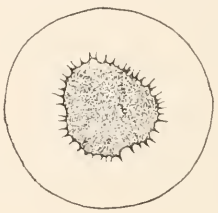

FIG. 4.

end presents somewhat the appearance of Fig. 3. The piece is collapsed, the margins are much folded and the only new tissue visible is the small area between the inrolled margins represented in the figure by the stippled portion.

These facts serve to introduce the chief point in the present consideration, viz., this: so long as there is no tension exerted upon this new tissue closing the ends it does not increase in surface-area, but mere differentiates into the typical structure of the body-wall. In other words the ends of the piece have no power in themselves to spread apart and thus initiate the formation of a new disc. What occurs in the typical course of regeneration is shown in Fig. 4. The inrolled margins are gradually spread apart while the thin membrane of new tissue uniting them — the stippled area in the figure — increases in area as they separate.

It is not difficult to show that this increase in area of the new tissue is due simply to the internal water-pressure. If the aboral end of the piece be prevented from closing or be punctured from time to time and thus the acclumulation of water in the enteron be prevented, the spreading of the oral end and the increase in area of the new tissue does not occur. The cut surfaces remain rolled inward and approximated as in Fig. 3, while the new tissue merely fills the spaces between them, but does not increase farther. A similar experiment affords the same result for the aboral end. In such cases the end retains the form acquired by inrolling of the margins (see Child, '03*a*, Figs. 3–5), differing from the stages figured only in that the crevices between the inrolled cut surfaces are closed by the new tissue.

Experiments of this kind were performed many times and always with the same result. Under conditions to be described later certain other regenerative processes may occur as usual in pieces which are kept open aborally, but in no case does the spreading apart of the cut surfaces at the oral end occur and in no case does the disc assume its typical form.

The increase in the area of the new tissue when under tension is not merely a stretching; as was noted above, attempts to stretch the tissue artifically always resulted in rupture. At the end of this extension of the new tissue its area is many times as great as at first and, what is more important, its thickness is as great as, or greater than before. Actual growth has without doubt occurred during the extension, indeed the conclusion is justified that the increase in area of the new tissue could not have occurred without growth; the attempts at artificial stretching leave little room for any other conclusion. If this conclusion is correct, then the growth of this new tissue is dependent on the tension to which it is subjected and not on any factor or condition existing in the tissue itself, except of course the power of growth. Unless the tissue is subjected to tension, growth ceases with the closure of the small spaces between the folds of the inrolled ends; when it is subjected to tension, within certain limits, it grows, *i. e.*, increases in quantity, number of cells, etc. Its increasing thickness and power of resistance to tension as differentiation occurs limit its growth, so that increase in area does not go beyond a certain point (Fig. 4).

In this case, as in the growth of new tissue between diverging cut surfaces described in the preceding paper (Child, '04), the localization and extent of growth is dependent, not on internal factors, but on simple mechanical conditions of tension.

The figures and the details of the description given refer to *C. solitarius*. The process of closure and distension in *C. membranaceus* differs in some details from this, though undoubtedly subject to similar conditions. It was shown in the preceding paper (Child, '04) that the new tissue in *C. membranaceus* is able to spread over larger areas and therefore to close larger openings than in *C. solitarius*, and moreover, that it is thicker and more resistant than in the latter species. Taking these facts into consideration we find that the process of closure and spreading of the ends is similar in both species. It is probable that the dependence of growth of the new tissue on tension would not have been so readily recognized in *C. membranaceus* if this form alone had been studied. The tissue after its formation is so resistant to tension that the distension of the piece is very commonly insufficient to bring about any considerable increase in the amount of new tissue after closure. Frequently, however, some growth after distension is recognizable, though it is usually relatively much less than in *C. solitarius*. This difference between these species indicates the importance of comparative study of related species in experimental work. Phenomena not readily interpreted in one species may be modified in another so that interpretation is without difficulty, and it is often possible, as in the present instance, to apply the interpretation obtained in the one case to the other, although its recognition in the first case would have been difficult or impossible.

Tentacle Regeneration in Relation to the Mesenteries.

Attention has already been called to the fact that tentacles do not regenerate in the absence of mesenteries (Child, '03*b*).

In regenerating ends representing levels near the oral end of the parent body the series of mesenteries is complete or nearly so ; farther aborally, however, mesenteries are present over only a larger or smaller portion of the circumference, according to the level. In the pieces from these regions a certain number of mes-

enteries regenerate and the series thus becomes complete. Still nearer the aboral end only a few mesenteries are present on the directive side, and finally only a single pair. In most of the pieces from this region regeneration of the mesenteries does not occur, probably because of the decreased reactive capacity of the tissues in this region, as has been suggested (Child, '03*b*). In certain pieces from this region the regeneration of tentacles corresponding to the intermesenterial chambers was observed to begin but never proceeded far and no mesenteries were regenerated (see Fig. 3, Child, '03*b*). The fact that tentacles never appear in such cases where mesenteries are absent indicates that their regeneration is dependent in some manner upon the presence of the mesenteries. In pieces where regeneration of the mesenteries occurs, regeneration of the tentacles never precedes or coincides in time with the appearance of mesenteries, but always follows. Various observations of others and my own upon other species of actinozoa indicate that this rule holds good not only for regeneration but for the normal development and for addition of new tentacles in adults. The new mesentery appears first, then the tentacle.

The interpretation of this "correlation" is not difficult if we accept the point of view suggested above. According to this it is evident that the mesenteries serve to localize the currents proceeding orally along the body-wall, *i. e.*, the current of one intermesenterial chamber is separated by the bounding mesenteries from that in the adjacent chamber. If the mesenteries were not present the current would strike the oral margin in a continuous circle and if it produced any effect at all would cause the outgrowth of the whole margin.[1]

The outgrowth of the whole margin, *i. e.*, the spreading of the disc, occurs to a certain extent in every case of regeneration and

[1] It is conceivable that in such a case the surface tension of the outgrowing margins might operate in such manner as to cause it to break up into a number of radiating masses of certain size. It is not impossible that the tentacles of certain cœlenterates are formed in some such manner whether the stimulus to growth be water-pressure or some other. This process might be compared to the breaking up of the margins of a drop of fluid into radiating cylindrical masses, which occurs when it is allowed to drop with force upon a smooth hard surface and "splashes." In this case we have a continuous cause with discontinuous effect. The close relation between the number of tentacles and the size, *i. e.*, the circumference, in such forms as *Hydra*, indicates the possibility of an explanation of this kind.

is probably due to the combined effect of the currents about the whole circumference, but the localized effect is much greater, giving rise to the long marginal tentacles. In its earlier stages the intermesenterial chamber formed between two regenerating mesenteries is simply a small blind pocket into which the water passing orally along the wall of the enteron is continually forced. In the normal animal there is a current passing aborally along the stomodæum (see Fig. 2), thus carrying the water out of the pocket, but in the earlier stages of regeneration the stomodæum and disc are absent ; *i. e.*, no cilia, or few, are present to produce a current in the aboral direction. On the other hand the current passing orally is not necessarily diminished by section. The consequence is that water is continually forced into the oral end of the intermesenterial chamber and strikes the body-wall with a certain pressure, but no means for its removal exists except as the incoming water displaces it.

It is clear without further discussion how the localization of the regenerating tentacles and their dependence upon the presence of mesenteries can be accounted for as a reaction to the tension produced by internal water-pressure.

Local Inhibition of Tentacle Regeneration.

The shape of the collapsed pieces after section and before closure and distension is various. Frequently some part of the oral end — as well as other regions — becomes involved in some fold or wrinkle in consequence of collapse. The new tissue binds the cut surfaces together however they may happen to lie, but if distension occurs within a few days the folds are soon obliterated because the growth of the new tissue corresponds to the tension. If, however, distension be prevented by keeping open the aboral end while the oral end is permitted to close, the new tissue uniting the cut surfaces thickens and becomes more resistant to tension in the course of a few days.

After two weeks or more, even if the piece is allowed to close aborally and become distended, the fold may persist for a long time, since union of the oral end has occurred in the folded condition and the new tissue is now so resistant that only very gradual change occurs. It is easy to see that when such a piece be-

comes distended with water after closure it may be impossible or difficult for the water to gain entrance into the intermesenterial chambers of the folded part and so this may fail to be distended; if it is not distended it will be compressed to a greater or less extent by the adjacent distended regions. In this manner it is possible to produce experimentally cases in which only a part of the circumference becomes distended with water after closure. In all such cases it is found that the tentacles appear only on the distended portions. If the infolded or compressed portions become distended later, tentacles appear.

Provided the aboral end is kept open long enough, tentacles may appear in those regions where the mesenterial chambers are sufficiently open to permit the entrance and circulation of water. Of course the formation of tentacles is much slower in this case than when the piece is permitted to distend, and they never attain very great length. Since the regeneration of the tentacles is very slow and does not proceed far, this method is less satisfactory than the first.

By both of these methods it is possible to bring about local inhibition of tentacle-regeneration. In some cases the folded or compressed region includes only one or two mesenterial chambers, and here only one or two tentacles are inhibited or delayed. In pieces with slightly irregular oral margins where closure is irregular there is often great irregularity in the tentacles, some appearing early, others late, but in every case it can be determined that the tentacles appear first on those parts where distension and the circulation of water is least hindered, and *vice versa*.

There is very rarely any difficulty in distinguishing the distended portions of the body from those that are collapsed or compressed; the former appear somewhat translucent and the surface is smooth; the latter are opaque and usually more or less wrinkled. Moreover there is a distinct difference in color between distended and collapsed portions, the former being always lighter in color, since distension stretches the body-wall and the stripes (in *C. solitarius*) are farther apart or the pigment granules (in *C. membranaceus*) less densely distributed. Thus distended parts can usually be distinguished at a glance from

those which are collapsed. It is possible to observe on the living specimens that the tentacle regeneration is more rapid in distended than in collapsed portions. Though it may not always be possible to determine exactly the causes that have operated in bringing about a particular case of local collapse or compression its presence can be recognized without difficulty, even before tentacle regeneration has begun.

A few examples selected from the numerous cases of this kind observed will serve to illustrate the subject. Fig. 5 represents a case in which one side of the oral region of a piece became folded inward during collapse and so failed to become distended when closure was allowed to occur. It will be observed that the new tissue closing the oral end does not form a rounded area but simply fills the crevices between the inrolled margins. The failure of the new tissue to distend is due to the fact that the aboral end was kept open until the new tissue had become thick

FIG. 5.

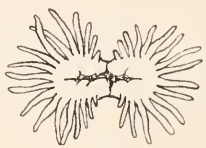

FIG. 6.

enough to resist the tension and to grow very slowly. It will also be observed that with approach to the infolded region the marginal tentacles decrease in size and finally cease. The region without tentacles represents a much greater portion of the circumference than is apparent in its contracted condition. The figure is simply a view of the oral end. A short distance from the end the fold disappears and the body is cylindrical in form. The fold was gradually obliterated in this piece and the missing tentacles finally appeared.

In Fig. 6 a similar case is shown, except that here two folds are present on opposite sides of the body. In the region of each of these folds the tentacles diminish in size and disappear as in the case of Fig. 5.

In these cases it is of course impossible to determine just why the water has failed to enter the infolded portions, but it is prob-

able that the mesenteries of these parts are so closely pressed together that the mesenterial chambers are almost obliterated and their communication with the general enteric cavity almost or quite shut off. After the other parts have once become distended these collapsed parts are compressed. Only as water gradually finds its way into them, either by diffusion or through small openings, will they become distended so as to permit the typical circulation.

A case obtained in a similar manner from a piece of *C. membranaceus* is shown in Fig. 7 (natural size). It could be seen without difficulty that the three regions bearing the long tentacles were distended while the intermediate regions were not or were only slightly. In the course of a few weeks the distension of all parts gradually become uniform and the delayed tentacles finally attained the same size as the others, though only after the others had ceased to elongate. Of course at a somewhat earlier stage than that figured not even minute tentacle-buds were present on the infolded regions though the tentacles on the distended regions were already well developed. In this case as in the others it is probable that in the infolded regions the mesenteries are so closely pressed together that little water enters the chambers.

FIG. 7.

These experiments have a certain bearing upon the question of localized pressure as a factor in determining the position of the marginal tentacles. It is evident that in the distended portions the circulatory currents must possess greater force and volume than in the collapsed portions, where they may indeed be absent or insufficient to act as stimuli. If the appearance of the marginal tentacles is due to the local pressure resulting from these currents it is easy to see why the tentacles appear earlier on the distended portion. In general internal pressure we can find no factor which can determine the position of these organs, and if they are determined by "protoplasmic" factors it is difficult to understand why they should appear earlier on the distended portions than on the others.

SUMMARY.

1. Except when the animal is contracted or collapsed the body-wall of *Cerianthus* is subjected to a certain degree of ten-

sion in consequence of the fact that the water in the enteron is under pressure.

2. The inward current along the siphonoglyphe is probably the means by which the internal pressure is established and maintained.

3. When the body is distended the œsophagus, with the exception of the siphonoglyphe and perhaps some grooves and crevices, must be closed.

4. When contraction occurs the water first issues from the aboral pore (in other actinians from the cinclides, etc.); then when the pressure is sufficiently reduced to permit it, the œsophageal walls are separated by muscular action and the remaining water issues from the mouth, often accompanied by mesenterial filaments. Thus the œsophagus is widely open only during extreme contraction.

5. The cilia on the entodermal surface of the body-wall produce a current flowing orally in each mesenterial chamber. The water passes from each chamber along the aboral face of the marginal tentacle, back on its oral face beneath the disc toward the stomodæum, probably into and out of the labial tentacles and aborally along the stomodæum. In all probability cilia along the sides or margins of the mesenteries force it further aborally.

6. The internal water-pressure plays a large part in form-regulation in *Cerianthus*. The general pressure affects the rapidity of growth wherever it may be taking place and it is possible that the local pressure exerted on the body-wall by the currents passing orally in each mesenterial chamber is the formative stimulus for the marginal tentacles.

7. Regeneration of tentacles is impossible unless mesenteries are present. The reason suggested for this is that in the absence of mesenteries there is no localization of the currents corresponding to the intermesenterial chambers, and, moreover, the water being unconfined between mesenteries, exerts less pressure on the inrolled oral end than if mesenteries were present.

8. Local retardation or inhibition of tentacle-regeneration can be brought about by preventing distension of a part or parts of the oral region.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO, September, 1903

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NOTES ON REGENERATION IN TUBULARIA CROCEA.

HELEN DEAN KING.

The following experiments on *Tubularia* (*Parypha*) *crocea* were made during the summer of 1903, at Woods Holl, Mass., while I was occupying a research room of the Carnegie Institution in the Marine Biological Laboratory. The work was done under the direction of Prof. T. H. Morgan to whom I am indebted for many helpful suggestions.

I. The Effect of the Earlier Closing of One End of a Long Piece of the Stem of Tubularia.

In experimenting on the European hydroid *Tubularia mesembryanthemum*, Morgan (10) allowed the ends of long pieces of the stem to close and then, after an interval of from one to eight hours, he cut the pieces transversely through the middle region so that two new cut surfaces were exposed (Fig. 1, *B*, *C*). As a result, the aboral development of the proximal piece *CD*, was hastened. In many cases a polyp appeared on the aboral surface, *D*, as soon as did a polyp on the oral end, *C*, and in a few pieces a hydranth developed at *D* as early as did the hydranth on the distal end of the anterior piece, *AB*. This result is explained by Morgan as follows: "When a piece is cut in two in the middle one, two, three or more hours after its ends have closed, the influence of the oral end is temporarily removed, and the aboral end, which now has a start on the new oral end, may gain the ascendency and be the first to produce a polyp. Often, however, the polarity of the piece is sufficiently strong to give the precedence to the influences acting on the oral end. When the two influences are equally balanced, two hydranths may simultaneously develop."

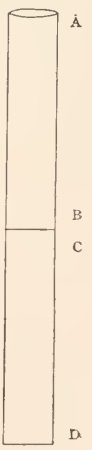

Fig. 1.

In repeating these experiments on the American hydroid,

Tubularia crocea, the hydranths already present at the anterior ends of the stems were removed by a transverse cut about 2 mm. behind the proximal circle of tentacles, and then pieces of stem varying in length from 30–40 mm. were cut off and the ends allowed to close. After an interval of from two to eight hours the stems were cut through the middle as in Fig. 1, *B*, *C*, thus producing a freshly cut surface (Fig. 1, *B*) at the aboral end of a distal piece, and also one at the oral end (Fig. 1, *C*) of a proximal piece. The results of this series of experiments are given in Table I. to IV. The first column gives the total number of individual stems operated upon; the second column shows the time that elapsed between the removal of the hydranth and the cutting of the stem through the middle; and in the following columns the results two, three, and four days after the operation are indicated. *Hy.* signifies the regeneration of a complete hydranth; *t. a.* indicates the formation of tentacle anlagen only; while *O* is used to indicate that no regeneration had taken place when the observations were made. The letters *A*, *B*, *C* and *D* refer to surfaces thus marked in Fig. 1; and the numbers in parentheses show the number of cases in which similar results were obtained.

TABLE I.

Number of Individuals.	Interval Between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
6	2 hours.	*A* ...*hy.* (6) *B*... *O* (6) *C*... *t. a.* (3), *O* (3) *D*... *O* (6)	*A* .....same. *B* *C*.... *hy.* (4), *t. a.* (2) *D*... *t. a.* (2), *O* (4)	*A* .....same. *B* *C*.....*hy.* (6) *D*... *hy.* (2), *t. a.* (1), *O* (3)

Owing, probably, to differences in the temperature of the water in which they live, regeneration in *Tubularia crocea* is much slower than in *Tubularia mesembryanthemum*. In the former species a new hydranth rarely develops until two days after the removal of the old hydranth, while in the latter species a new hydranth frequently regenerates in the course of twenty-four hours. In all of the experiments in this series, as shown in the above table, a polyp formed on the oral end, *A*, of the anterior

piece, *AB*, before one developed on the oral end, *C*, of the proximal piece, *CD*. This result might, of course, be expected as the oral end, *A*, closed two hours before the end, *C*. In no case did a polyp develop at the aboral end, *D*, of the proximal piece as soon as one formed at the oral end, *C*. A start of only two hours, however, is sufficient, in most cases, to cause the formation of a hydranth at *D* before one develops at the aboral end, *B*, of the distal piece, *AB*.

The results obtained when the intervals between the cuttings were four, six and eight hours are given in Tables II. to IV.

TABLE II.

Number of Individuals.	Interval between Cuttings	Result in Two Days	Result in Three Days.	Result in Four Days.
10	4 hours.	A ... hy. (6), O (4); B ... O (10); C .... t.a. (1), O (9); D ... O (10)	A ... hy. (6), t.a. (4); B ... O (10); C .... hy. (2), t.a. (5), O (3); D ... t.a. (1), O (9)	A ... hy. (10); B ... t.a. (2), O (8); C .... hy. (7), t.a. (3); D ... hy. (1), O (9)

TABLE III.

Number of Individuals.	Interval between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
8	6 hours.	A ... hy. (3), t.a. (3), O (2); B ... O (8); C .... hy. (1), O (7); D ... O (8)	A ... hy. (6), t.a. (2); B ... O (8); C .... hy. (5), t.a. (3); D ... t.a. (4), O (4)	A ... hy. (8); B ... O (8); C .... hy. (8); D ... hy. (4), O (4)

TABLE IV.

Number of Individuals.	Interval Between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
12	8 hours.	A ... hy. (6), t. a. (4), O (3); B ... O (12); C .... O (12); D ... O (12)	A ...hy. (12); B ...O (12); C .... hy. (10), t. a. (2); D ... hy. (2), t. a. (3), O (7)	A ...hy. (12); B ...O (12); C ....hy (12); D ... hy. (5), O (7)

There is a great similarity in the results of these experiments. In all cases a hydranth formed on the oral end, *A*, of the anterior piece before one developed at any other cut surface, and the rate of development of a polyp from the aboral surface, *D*, was more rapid than that from the aboral surface, *B*, even in those cases in which *D* had only two hours start over *B*. In no case, however, did a polyp form at *D* before one developed at *C*, even in the experiments in which *D* closed eight hours before *C*. As was the case in the experiments made by Morgan, an interval of eight hours between the two cuttings has, apparently, no more effect on the regeneration than has an interval of only two hours. The formation of a polyp at the oral end, *C*, of the proximal piece, *CD*, does not prevent the early development of a polyp at the aboral end of the same piece, and in some cases there is only a few hours interval between the formation of the two hydranths. The earlier development of a hydranth at *A*, however, seems to check the formation of a hydranth at the aboral end, *B*, of the distal piece for some time, as in no case did a hydranth develop at *B* until five days after the experiment began. This difference in the rate of development at *D* and at *B* cannot be due to a difference in the lengths of the pieces, because, in making the experiments, the stems in all cases were cut as nearly as possible through the middle and any difference in the lengths of the anterior and of the proximal pieces would be too slight to have any appreciable influence on the result. The earlier closing of the aboral end, *D*, of the proximal piece, *CD*, evidently counterbalances to some extent the influence of the oral end, as suggested by Morgan. As a result, the development of a polyp at *D* is hastened somewhat, although in no case is a hydranth formed here before or as soon as one develops at the oral end of the piece.

The effect of the earlier closing of the aboral end of long pieces of the stem, in both *Tubularia mesembryanthemum* and in *Tubularia crocea*, is to hasten the development of the aboral surface. The influences that bring about this result are apparently not as strong in the latter species as in *Tubularia mesembryanthemum* where the aboral development may be hastened so much that polyps develop simultaneously at both ends of the piece.

This seeming difference between the two species may possibly be due to the fact that *Tubularia crocea*, which lives in cold water, regenerates very slowly and, therefore, comparatively slight differences in the rate of regeneration at the oral and aboral ends of the stem can be readily noted. *Tubularia mesembryanthemum*, on the other hand, lives in much warmer water and its regeneration takes place so quickly that it is difficult to detect slight differences in the rate of development of the polyps at the cut oral and aboral surfaces.

In a variation of the above experiment, a piece of silk thread was tied tightly around the stem about 2 mm. below the hydranth, and another piece was tied about 30–40 mm. below the first. Both ends of a long piece of stem were, therefore, closed at practically the same time in such a way that no regeneration was possible from either end of the piece. After the ends had been tied, the stem was cut transversely through the middle as in Fig. 1, *B*, *C*, in order to ascertain whether subsequent regeneration from the cut surfaces, *B*, and *C*, would be hastened in comparison with the rate of regeneration from similar surfaces of pieces of stem of the same length, cut at the same time, but not closed artificially at one end. The control pieces of stem were kept in the same dishes with those used in the experiment, and both sets, therefore, were under the same external conditions.

Eight long pieces of stem were used in this experiment. Two days after the operation, tentacle anlagen had appeared at the cut ends of all of the sixteen pieces, but they were not as well developed on the aboral end, *B*, of the anterior piece as they were on the oral surface, *C*, of the posterior piece. At this time there was no indication of any regeneration at the aboral surface of the anterior piece in the control set of stems, although in some cases complete hydranths, in other tentacle anlagen, were present on the oral end of the proximal pieces. On the third day after the operation, polyps were found on the oral end, *C*, of all of the proximal pieces, both in the control and in the tied stems. The development from the aboral surface, *B*, of the anterior pieces, however, did not keep pace with that at the oral end, *C*, of the proximal pieces, as at this time only two of the pieces of stem tied at one end had produced hydranths at the aboral sur-

face, *B*, the rest had, as yet, developed only tentacle anlagen ; in the control stems, no development from the aboral surface, *B*, had taken place in any case.

It is seen from the above experiments, that the development of a hydranth at the oral end of a piece of the stem of *Tubularia crocea* is not hastened by artificially closing the aboral end. Tying the oral end of a distal piece of the stem, however, hastens the development of the aboral end of the piece as compared with the development that takes place from the aboral surface of a piece of stem of similar length that is not closed at the oral end, as Driesch has shown. This result also agrees with that obtained by Loeb (7) in experiments in which he stuck the oral end of pieces of the stem of *Tubularia mesembryanthemum* in fine sand, leaving the other end freely surrounded by water. He found that "Durch Hemmung der Polypenbildung am oralen Ende kann man also die Polypenbildung am aboralen Ende beschleunigen."

In a third set of ten experiments, hydranth bearing stems about 30 mm. in length were removed from the colony and kept until a polyp formed on the cut aboral end. The time required for the development of these aboral polyps varied from three to five days in different cases. After all of the pieces had developed hydranths at the aboral end, each stem was cut transversely through the middle as in Fig. 2, *B*, *C*. The object of these experiments was to ascertain whether the presence of a hydranth at the aboral end, *D*, of the proximal piece, *CD*, would alter the polarity of the piece and thus prevent or retard the development of a hydranth at the oral end, *C*. The stems were cut through the middle region on June 19. Not until June 22 were there any indications of a development of a hydranth at the oral surface, *C*, and then only faint traces of tentacle anlagen were found in three stems. At this time, no development had taken place from the aboral surface, *B*, of any of the anterior pieces, *AB*. For control experiments, pieces of stem about 30 mm. in length were cut through the middle as in Fig. 1, *BC*, at the same time that the transverse cuts were made across the stems bearing a hydranth at each end, and on June 22, well developed hydranths were present at the oral end of all of the proximal pieces. On

June 23, three of the ten proximal pieces of stem bearing aboral hydranths had also developed hydranths at the oral end; while the oral end of two other pieces of stem showed well developed tentacle anlagen which developed into hydranths on the following day. No further changes took place in any of the pieces although they were kept for some ten days longer.

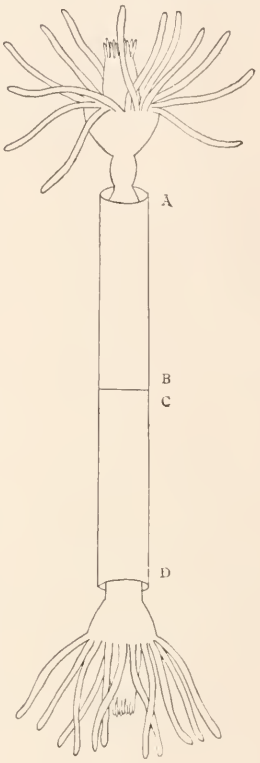

FIG. 2.

It is seen from the above experiments, that the presence of a hydranth at the aboral end of a piece of the stem of *Tubularia* delays, but does not prevent, the development of a hydranth at the oral end of the piece. This result cannot be due simply to the fact that the proximal end of the piece was closed by the presence of the aboral polyp, because, in the previous set of experiments, it was shown that closing the aboral end of a piece of stem by tying does not delay the development of a polyp at the oral end. It seems probable that the polarity of the piece was changed, for a time at least, by the presence of a hydranth at its aboral end and, therefore, the influences for hydranth formation at the freshly cut oral surface were not strong enough to bring about the development of a polyp for some days.

II. Experiments on Branching Stems.

The following series of experiments were made in order to ascertain whether the development of a hydranth on the oral end of a stem will influence the rate of development of a hydranth on the distal end of a long or of a short piece of a branch, and also to determine what conditions are necessary in order that the formation of a hydranth at the one place will prevent the formation of a hydranth at the other.

Series 1. — On June 24, twenty experiments were made in which a branch was cut off about 1 mm. from its origin in the stem, and then the anterior end of each stem was removed by a transverse cut leaving a piece from 10–20 mm. above the place of union with the branch (Fig. 3). In one case, a hydranth developed on the cut end of the branch two days after the operation, and at the same time a polyp also formed on the oral end of the main stem which in this instance was 20 mm. in length above the origin of the branch. In all other individuals at this time tentacle anlagen had formed at the oral end of the stem, but there was no indication of the development of a hydranth at the cut end of any of the branches. On June 27 hydranths were found at the oral end of all of the stems and also on the distal end of four branches; all the remaining branches had well developed tentacle anlagen excepting one which showed no signs of regeneration during the course of the week that the hydroids were kept. In this set of experiments, therefore, with the exception of the one case noted, regeneration of a hydranth took place at the distal end of the long stem before a polyp formed at the oral end of the short branch.

FIG. 3.

The results of this set of experiments might possibly be considered to be due to the fact that the longer piece exerted some kind of an influence over the shorter piece that would tend to alter the polarity of the shorter piece and thus retard development from its cut oral surface. That a larger piece of a hydrozoa can influence the polarity of a smaller piece is shown unquestionable in grafting experiments that I made on *Hydra viridis* (King, 6) in which the larger component of the graft either absorbed the smaller component or formed a permanent union with it. In the latter case, the polarity of the smaller piece was completely reversed, if necessary, in order that a structure might regenerate on its cut surface that would produce a normal polyp. Another factor that might, possibly, cause a delay in the development of a hydranth from the cut surface of the shorter piece is the length of the piece. Morgan has shown that in

branching stems a short piece of a branch or of a stem regenerates as does an isolated short piece, *i. e.*, the region of the tentacle anlagen is reduced and the rate of development of a hydranth is much slower than that of a hydranth from the cut end of a long piece of stem. Both of these factors may help to bring about the delay in the development of a hydranth from the oral end of the shorter piece in all of these experiments with branching stems in which there is a marked difference in the length of the stem and of the branch.

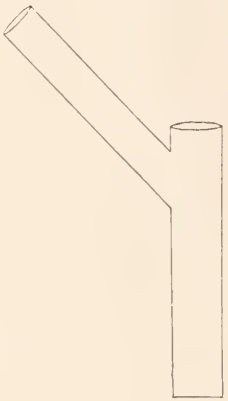

FIG. 4.

Series 2. — Seventeen branching stems from different colonies were cut so that the anterior portion of the stem above the place of union with the branch was only about 1 mm. in length, while the length of the branch varied in different cases from 8–25 mm. (Fig. 4). Two days after the operation, hydranths had developed at the cut ends of thirteen branches, and well developed tentacle anlagen were found at the oral ends of the other four branches. No hydranths were found at this time at the oral end of any of the stems, and tentacle anlagen were only faintly defined in some few cases. On the next day, hydranths had developed at the distal end of all of the branches, but only five stems bore hydranths at the oral end. In this set of experiments the development of a hydranth took place more rapidly from the cut end of the long branch than from the oral end of the main stem.

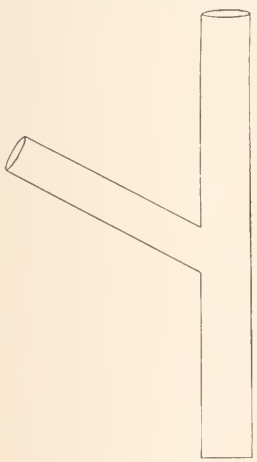

FIG. 5.

Series 3. — Twelve experiments were made in which the anterior end of the stem and the distal end of the branch were cut off so that the length of the branch and of the stem above the place of union was practically the same, varying in different cases

from 8–20 mm. (Fig. 5). Two days after the operation, there were well developed hydranths on the ends of three branches and only tentacle anlagen on the oral ends of the corresponding stems. In two cases hydranths had developed on the end of the branch and also on the cut surface of the stem; while in the other cases only tentacle analgen were found, and they were equally well-developed on the branch and on the oral end of the stem. During the following two days, hydranths formed on all of the cut ends, sometimes the hydranth developed on the end of the stem before it did on the branch, and sometimes the hydranth appeared first on the branch. As a general result of this series of experiments it can be stated that hydranths develop at about the same rate when both the branch and the anterior portion of the stem are approximately the same length.

Series 4. — In the previous set of experiments both the branch and the anterior portion of the stem were of considerable length and both developed at about the same rate. In order to see if similar results would be obtained if the pieces were very short, fifteen experiments were made in which the branch and the stem were cut off about 1 mm. above their point of union (Fig. 6). When the hydroids were examined three days after the operation, hydranths were found on the oral end of the stem and not on the cut end of the branch in five cases; while in four hydroids, polyps had developed on the branch and not on the oral end of the stem; in the remaining six cases, hydranths were present at the distal end of both branch and stem.

FIG. 6.

In those cases in which one or the other cut surface failed to develop a hydranth, the cœnosarc appeared to be entirely with drawn from this part. When these stems were examined under the microscope, the streaming of granules in the interior cavity was visible only in the proximal part of the main stem and in the part of the stem or branch that had regenerated. In the cases in which hydranths formed at both cut surfaces, the streaming of granules was found in all parts of the stem and also in the branch.

In this set of experiments, regeneration seemed to take place

with equal rapidity from the cut oral surface of the branch and of the stem, as was the case in the experiments described under series 3. If the pieces above the place of union of the branch and stem are of approximately the same length, no matter how long or how short they may be, the influences for hydranth formation appear to be alike in both, and one piece has, seemingly, no effect on the other. Where the cœnosarc is withdrawn entirely from one part of the hydroid, as was noted in some few cases in the last set of experiments, no regeneration of the piece is possible.

In experimenting on *Tubularia crocea*, Morgan (9) found that if he cut off both the main stem and the branch a little above the place of union, the results varied considerably in different cases. In some instances he obtained the regeneration of a hydranth on the branch and not on the oral end of the stem; in other cases a polyp formed only at the oral end of the main stem; and in still other individuals regeneration took place from the cut surfaces of both branch and stem. It seems probable that the lack of uniformity in these results can be attributed to a difference in the relative lengths of the branch and of anterior portion of the stem above the point of insertion of the branch. It is, of course, impossible to cut the branch and stem at absolutely equal distances from the place of their union, and in those cases in which regeneration from one cut surface took place before it did from the other, there may have been just enough difference in the lengths of the pieces to bring about the earlier regeneration of a hydranth on the cut oral surface of the longer piece.

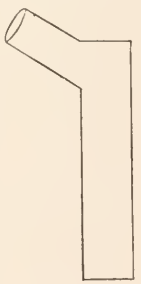

FIG. 7.

Series 5. — In twenty-eight cases the stem was cut off transversely just above the origin of the branch as shown in Fig. 7. The oral end of the branch was then removed leaving a piece, from 3 to 5 mm. in length, still attached to the stem. The object of these experiments was to see whether the regeneration of a hydranth at the oral end of the stem could be entirely prevented by this means. Two days after the operation, hydranths had developed at the cut end of the branch in nine of the hy-

droids and well-developed tentacle anlagen were present on the other branches; there was no indication of a regeneration at the oral end of any of the stems. The next day all of the branches bore hydranths, and in but one case had any regeneration taken place at the oral end of the stem. In this instance, the stem extended about 0.5 mm. above the place of insertion of the branch and a considerable amount of red pigment had collected at its extreme oral end. In the course of forty-eight hours more a polyp formed on the oral end of this stem but no regeneration took place at the oral end of any of the other stems, although they were kept for over a week.

Series 6.— Sixteen experiments were made in which the branches were cut off very close to their origin on the stem. The oral end of the stem was then removed leaving a piece about 5 mm. in length above the origin of the branch, in order to see whether the formation of a hydranth at the oral end of the stem would prevent or merely delay the formation of a polyp at the place where the branch was removed. In all cases the wound in the side of the stem healed over very quickly and, although the hydroids were kept alive for a number of days, no regeneration of any kind took place at the point of injury.

Series 7. — In ten cases the entire branch was removed from the stem, but the old hydranth at the distal end of the stem was not cut off. The result was the same as in the previous set of experiments, as the cut surface was very soon covered over and no subsequent regeneration took place from it.

Series 8. — In sixteen cases where long pieces of stem bore from two to four branches, the anterior end of the stem and the apical end of each branch were removed by transverse cuts leaving the lengths of the branches approximately the same as that of the stem above the origin of the most anterior branch. The experiments were made to see if there is any difference in the relative rate of regeneration of the anterior branches and of the proximal ones. There was no uniformity whatever in the results of this set of experiments. In some cases a hydranth regenerated on a posterior branch before it did on the oral end of the main stem; and in other cases all of the branches produced hydranths at the same time that one developed at the oral end of the stem.

III. The Regeneration of Short Pieces of the Stem of Tubularia.

It was first noted by Bickford (1), and later confirmed by Driesch (2) and by Morgan, that small pieces of the stem of *Tubularia* about 1 mm. in length are capable of regenerating. In a recent paper, Hargitt (5) states that he was unable to obtain any regeneration from pieces of the stem of *Tubularia crocea* and of *Tubularia tenella* that were as much as 3–4 mm. in length. This result was probably due to the poor condition of the stems when the experiments were made. Small pieces of the stem of some other hydroids, do not appear to possess as great a power of regeneration as *Tubularia*, for Gast and Godlewski (4) have found that pieces of the stem of *Pennaria cavolinii* about 1 mm. in length never produce hydranths and pieces 2 mm. in length regenerate hydranths but rarely. Bickford's experiments on small pieces of the stem of *Tubularia tenella* show that, in this species, regenerative processes are not restricted to any special region of the stem, and also that such short pieces tend to form one complete hydranth rather than to produce double abnormal structures.

In experimenting on *Tubularia mesembryanthemum*, Driesch (2) found that of 82 short pieces of stem, 5 formed a single proboscis, 26 formed a double proboscis, and the remaining 51 pieces produced hydranths. These results agree with those of the earlier experiments of Bickford. In a later paper, Driesch (3) states that at the oral end of the stem one seldom gets a whole hydranth, but usually a single or a double proboscis; from the middle zone hydranths usually develop; while from the aboral end of the stem, these structures are rarely produced. This difference in the kind of regeneration from the various parts of the stem Driesch attributes to the situation of the small piece in the original individual and to the different distribution of the hydranth-forming pigment in the cœnosarc of the different parts. Since Morgan and also Stevens (11–12) have proven the fallacy of the hypothesis of "red formative stuff" in *Tubularia*, this portion of Driesch's explanation is, of course, no longer tenable.

Morgan (8–10) has made an extended series of experiments with small pieces of the stem of both *Tubularia mesembryanthemum*

and of *Tubularia crocea*. He finds, as did Driesch, that pieces about 1 mm. in length from the region immediately behind the old hydranth usually die, even when longer than pieces from a more proximal region that regenerate. When this distal region does regenerate, it produces a greater number of single proboscides than of other forms, a result that might be expected as this part of the stem ordinarily goes into the proboscis of the new hydranth when a long piece of stem is regenerating.

In another set of experiments, Morgan cut pieces of the stem of *Tubularia mesembryanthemum* into a series of small pieces about 1 mm. in length in order to observe the behavior of consecutive pieces from one stem and to compare the results with those obtained from similar pieces cut from other stems. His tables do not show any very definite results although there seems to be a certain similarity in the behavior of pieces of the same stem, and the incomplete structures are found most frequently at the distal end of the stem.

At the suggestion of Professor Morgan, I repeated his experiments, using *Tubularia crocea*, in order to furnish more data from which definite conclusions could be drawn. In making the experiments the old hydranths were removed and the distal part of the stem was cut into consecutive pieces about 1 mm. in length. The pieces were than laid in rows on the bottom of flat dishes filled with fresh sea water. For the sake of brevity the following abbreviations are used in the tables given: hy. = complete hydranth without any stalk; hy. + stalk = hydranth with a short stalk that has been formed by a withdrawal of the cœnosarc from the perisarc; hy. + stem = hydranth with a stem attached to the perisarc; pb. = single proboscis; d. pb = double proboscis; reprod. = reproductive organs. The results tabulated are from observations made three to four days after the operation.

TABLE V.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb. with no tentacles.	6	pb.
2	d. pb.	7	d. pb.
3	pb.	8	hy. + stem.
4	d. pb. + reprod.	9	d. pb.
5	pb.	10	d. pb.

Table VI.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb.	6	dead.
2	d. pb.	7	pb.
3	d. pb.	8	pb.
4	dead.	9	dead.
5	d. pb.	10	dead.

Table VII.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	d. pb.	6	d. pb.
2	pb.	7	d. pb. + reprod.
3	pb.	8	pb.
4	pb.	9	hy. + stem.
5	hy.	10	pb.

Table VIII.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	dead.	6	pb.
2	d. pb.	7	pb.
3	d. pb.	8	hy. + stalk.
4	pb.	9	pb.
5	pb. + reprod.	10	d. pb.

Table IX.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	dead.	6	pb. + reprod.
2	pb.	7	pb.
3	pb.	8	hy. + stem.
4	hy. + stem.	9	pb.
5	pb.	10	dead.

Table X.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb.	6	pb.
2	d. pb.	7	hy. + stem.
3	d. pb.	8	pb.
4	d. pb. + reprod.	9	hy.
5	pb	10	dead.

As was the case in the experiments made by Morgan, the results for corresponding pieces of different stems are far from uniform, and it is not possible to determine what kind of a structure will be produced by a small piece from a given region of the stem. It is evident that the power to form either complete or double structures is present throughout the stem, and just what conditions are necessary to produce certain structures have not, as yet, been fully determined. Morgan has suggested that possibly the factors in determining the kind of regeneration are (1) the smallness of the piece, (2) the differences in the region of the original stem from which the pieces came (this factor had been previously suggested by Driesch), (3) the age of the piece, as the younger the stem the more likely it would be to form incomplete structures.

According to this set of experiments short pieces, no matter from what part of the stem they are taken, are more liable to produce proboscides than to form hydranths. When the latter structures appear they are usually produced by the more proximal pieces of the stem, the distal end of the stem showing a great tendency to produce incomplete structures. These results are very similar to those obtained by Morgan on *Tubularia mesembryanthemum*.

In order to ascertain whether the double structures that are so often obtained in such experiments are produced because the small pieces of the stem are open at both ends and not because there is insufficient material in the piece to produce a complete hydranth, Morgan tied one end of a short piece with silk thread, and found that, under these conditions, double structures are never produced. Later he planted short pieces of stems in rows in sand so that one end was buried and the other freely surrounded by water. In two instances only was a double proboscis formed, in all other cases single structures, either incomplete or whole, more often the latter were produced.

In repeating these experiments of closing one end of a short piece of the stem of *Tubularia* in order to ascertain the effect on the kind of structure produced, the following method was used: Shallow, flat dishes were covered on the bottom with a layer of paraffine about one fourth of an inch in thickness, and then, with

the blunt end of a large needle about the diameter of a tubularian stem, rows of holes were made in the paraffine about 0.5 mm. in depth. The dishes were filled with water and the long pieces of stems were put in them and cut into consecutive pieces about 1 mm. in length. One end of each piece was then inserted in a hole that was just large enough to receive and hold it upright. This method has the advantage that the inserted end of the piece of stem is in contact with the paraffine and cannot become free if the experiment is properly done. The results of this series of experiments are summarized in the following tables in which the abbreviations used are the same as those previously employed.

TABLE XI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb.	6	pb.
3	pb. + reprod.	7	dead.
4	dead.	8	pb.

TABLE XII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	hy.	7	pb.
4	dead.	8	dead.

TABLE XIII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	dead.	6	pb. + reprod.
3	d. pb.	7	pb.
4	dead.	8	pb.

TABLE XIV.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	hy.	5	dead.
2	pb.	6	dead.
3	pb.	7	pb.
4	pb.	8	dead.

TABLE XV.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	dead.
3	pb. + reprod.	7	dead.
4	pb.	8	dead.

TABLE XVI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	pb.	7	pb.
4	hy. + stem.	8	dead.

TABLE XVII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb. + reprod.	6	dead.
3	pb.	7	dead.
4	pb. + reprod.	8	dead.

TABLE XVIII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	dead.	5	dead.
2	pb.	6	hy. + stalk.
3	pb.	7	pb.
4	pb.	8	dead.

TABLE XIX.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb. + reprod.	6	dead.
3	hy. + stalk.	7	hy. + stem.
4	pb.	8	hy. + stem.

TABLE XX.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	hy. + stem.	7	pb.
4	dead.	8	dead.

TABLE XXI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced
1	pb.	5	dead.
2	pb. + reprod.	6	pb.
3	pb.	7	pb.
4	hy. + stem.	8	dead.

TABLE XXII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	dead.	6	hy. + stalk.
3	pb. + reprod.	7	pb.
4	pb.	8	dead.

The results of these experiments confirm those obtained by Morgan in every respect, as double structures were produced very rarely, only one being obtained in the entire series of experiments. There seemed to be no distinctive individual differences in the pieces of stem as regards the structures produced. In only one case (Table XIX.) were as many as three hydranths produced, while in the tables given by Morgan for *Tubularia mesembryanthemum*, whole series of pieces from the same stem produced hydranths.

If a comparison is made between the results shown in Tables XI. to XXII. and those shown in Tables V. to X., the most noticeable difference is that a very much greater number of double structures were produced when short pieces of the stem were lying on their sides during the process of regeneration. In both sets of experiments single proboscides were the structures most frequently produced, and very little individual difference could be detected in the stems regarding the kind of structure that they would tend to produce.

The development of small pieces of the stem of *Tubularia* standing on one end is considerably slower than that of similar pieces lying on one side. In the latter case, development takes place in about two days; while in the former case the various structures never appear under three days, and usually not under four or five days. Many pieces, usually those nearer the proxi-

mal end of the stem, die when the pieces stand on one end. This result may be due to the fact that the conditions under which regeneration takes place are not as favorable when the end of a small piece of stem is closed by contact with some foreign substance, as when the piece lies on one side and the cut ends are allowed to close in a normal manner.

BRYN MAWR COLLEGE,
Bryn Mawr, Pa., March 23, 1904.

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RESEARCH SEMINAR OF THE MARINE BIOLOGICAL LABORATORY FOR THE YEAR 1903.

The Research Seminar, begun in 1903, was devoted chiefly to informal reports and discussion of work carried on at the Laboratory. It found abundant support and was continued for some weeks after the regular courses ended.

The range of subjects reported upon contrasts strikingly with that of earlier years, when embryology was the main field of productive research at the Laboratory.

The following is a list of the subjects presented, with brief summaries:

July 13. **The Anatomy and Embryology of Pecten tenuicostatus.** By GILMAN A. DREW.

Drawings illustrating the structure and development of this form were exhibited and points of interest were discussed. Much of the discussion concerned the nervous system, the circulatory system, the formation of organs, and the excessive development of the posterior portion of this animal.

The visceral ganglia, which supply nerves to the greater part of the body, are very complex, and the different structural regions are very well marked. The pallial nerves have taken on ganglionic structure probably because of the many tentacles and eyes on the mantle margin that they supply. The cerebral and pedal ganglia are small, corresponding to the reduced condition of the anterior portion of the body. Octocystic canals opening on the surface of the body are present.

The circulatory system comprises a definite system of blood channels, but they are probably not lined with endothelium, so that there is no sharp distinction between blood and lymph.

July 15. **The Origin and Relationships of the Rock Pigeons as Revealed in their Color-Patterns.** By C. O. WHITMAN.

The wild rock pigeons present two very distinct color-patterns — (1) the chequered type, and (2) the barred type.

Two black wing-bars on a gray ground have always been held to be the more primitive pattern, and birds of this pattern are supposed to represent the typical *Columba livia*.

The form with black chequers evenly distributed over the wing and back, although once named *C. affinis*, as a distinct species, was regarded by Darwin as a variety derived from the two-barred rock, and his opinion has stood undisputed.

It appears from a comparative study of many species of wild pigeons, and from a study of the variations in domestic species, that the relationship is just the reverse : *C. affinis* is the original rock dove, and *C. livia* is the derived type. Domestic pigeons have come from both sources.

Columba affinis, however, is not the most primitive form among the wild pigeons. It was derived from a more ancient type, best preserved in the turtle doves (*Turtur orientalis* and *Turtur turtur*). In the turtle dove type each feather has a dark center and light edge.

The turtle dove pattern is at the same time a general avian pattern.

The turtle type and the rock type coexist in some forms (*e. g.*, *Phaps chalcoptera*).

The two-barred pattern of *Columba livia* was reached in the simplest way by an even reduction of the dark pigment, which would result, at one stage, in leaving remnant spots on the long coverts and the secondaries.

The process of reduction has run in *one direction* in many, if not all pigeons, and present species have reached different stages, varying all the way from a uniformly spotted condition to four, three, two, one or part of one bar, or no bars.

It appears to be impossible to reverse the direction of evolution, and to advance from one or two bars to a complete or even a partial chequered state.

The white-winged pigeon (*Melopelia leucoptera*) has no black spots or bars on the wing in the adult plumage, but unmistakable evidence of their former existence is seen in *structural* imprints still left on many of the coverts, and in vestigial traces of spots in a few of the juvenal feathers.

July 17. **Effects of Light-rays on an Ant.** By ADELE M. FIELDE.

The species used for the experiment was *Stenamma fulvum*, a myrmicid ant. Five queens and about two hundred workers were kept for ten months in each of five artificial nests, one nest roofed with transparent glass, one with opaque glass, one with glass transmitting only red and green rays, and two with glass transmitting only rays of shorter wave-length than blue. These nests were exposed to like temperature, and the ants were fed on the same foods. In all the nests the young passed safely through the egg, the larval and the pupal stage, and reached active life, proving that the cause of the usual hasty withdrawal of the inert young from daylight does not lie in any injury by any of the rays of the spectrum.

The ants are blind to all rays of light other than the ultra-violet, and they avoid the ultra-violet rays in proportion to the intensity of the illumination. They often congregated with their young in the spots where the illumination from the red and green rays was most intense. Orange glass which excludes most of the ultra-violet rays may be used for roofing their artificial nests, and then the ants may be studied with the certainty that they behave as if they were in darkness.

At the end of ten months marked ants from each of the five nests were introduced into each of the other four nests, where they were amicably received, and it was thus determined that exposure to any ray of light in the spectrum does not affect the odor whereby the ants recognize one another.

July 20. **Further Experiments in the Embryology of the Chick.** By F. R. LILLIE.

First, three cases in which the cerebral hemisphere of the right side was destroyed at an early stage, and which resulted, after farther growth of the embryo for four or five days, in displacement of the wing rudiments of the opposite side. This displacement was the same in all cases and was never found in normal embryos. The conclusion was that there is some trophic relation between the higher brain centers of one side and the embryonic tissues, especially muscular tissues, of the opposite side; this be-

ing expressed in the case under consideration by the abnormal growth of the wing. The sections of these embryos show that the parts of the brain posterior to the cerebral hemispheres on the defective side were less developed than on the uninjured side, owing to the absence of descending tracts from the cerebrum of that side. This defect disappeared in the region of the medulla, from which it may be concluded either that the tracts had descended no farther, or that approximately half had decussated. The trophic effect, if any, would be indirect, through the intermediation of the motor neurons of the chord. Subject still under investigation.

A second series of experiments was made involving destruction of the hind end of the embryo, showing, first, that there is no regeneration, and second, that the uninjured embryonic parts developed precisely as they would have done in a normal embryo. Thus, if but a single leg-somite remained, a rudiment of the leg was formed in a developing embryo; but if all the leg-somites were destroyed no rudiment appeared. Absence of the allantois was noted in such embryos.

Other modifications of internal organs were described.

July 22. **Variation and Selection in Saturnid Lepidoptera.** By Henry Edward Crampton.

An account was given of a study upon variation and its relation to elimination in *Philosamia cynthia*, as well as of additional studies which have been prosecuted during the past five years upon the same, and other problems in the case of *Cynthia* and other saturnid moths. The first points determined were: (1) That when normally formed pupæ which died before metamorphosis, were compared sex by sex with pupæ which lived through the metamorphosis, the two groups were markedly different in several characters, as regards the typical condition as well as the variability with reference to such typical condition. Thus pupal elimination and variation were shown to be related. (2) A comparison of the pupæ which metamorphosed perfectly with those which formed more or less imperfect moths revealed a second period of elimination which again proceeded hand in hand with variation. Thus "secular" and "periodic" selection, so-called

by Pearson, were proven for *Cynthia* of this year (1898–1899). Annual series of the same species exhibited the same phenomena. Three lots of *Cecropia* have been likewise examined, in much greater detail (28 characters), and these also show both kinds of selection.

An enquiry into the occurrence of reproductive selection grew naturally out of the foregoing. Two annual series of *Cynthia* (more than two hundred matings) and two annual series of *Cecropia* (130 and 125 matings respectively) have afforded the basis for the following conclusions: Pupal characters of mating males, or females, when compared with similar characters of the non-mating males, or females, prove to be different in type and restricted in variability. Thus a third period of selection is shown to be correlated with variability.

In discussing the nature of the selective processes, reasons were given for regarding the correlations between the several characters as the real bases for the action of eliminating processes, and not the individual characters themselves.

Further studies, now in progress, were briefly described. These deal with the phenomena of correlation between characters of the individual in its successive larval, pupal and imaginal conditions; with inheritance in pure breeds, and in mixed breeds; and with the correlation of certain physiological reactions with structural characters.

July 24. **White Feathers.** By R. M. Strong.

The few references in the literature of feather coloration to the causes of white in feathers state that the whiteness is due to the presence of air in the feather substance. This is misleading, for the larger proportion of the white effect is produced by the barbules which do not have air-spaces of significant size. The barbules are white for the same reason that powdered ice or glass and other transparent substances in a fine state of division appears white.

July 24. **Nervous Regulation of the Heart of Venus Mercenaria.** By R. A. Budington.

The details of the nervous control of the heart of vertebrates have been worked out in more and more minuteness since the time when

attention was first called to the matter by the Weber brothers in 1846. A similar relation of the nervous system and heart has been shown to hold among the invertebrates also, notably through the work of Conant and Clark on arthropods, of Ransom, Straub, and Yung on mollusca. The last-named worker has described inhibition of the heart of lamellibranchs on stimulation of the visceral ganglion, but no graphic records have been published illustrating experimental investigations of this kind.

In this seminar experimental work on the heart of *Venus mercenaria* was described, and kymograph tracings exhibited, which seem to warrant the following conclusions:

1. Normal rate and character of the heart-beat varies widely with different individuals.
2. Partially exhausted hearts show extreme irregularities.
3. Electrical stimulation of the visceral ganglion causes arrest of the heart.
4. Stimulation of the cerebral ganglion produces no effect.
5. Stimulation of nerves passing from the visceral ganglion to the heart gives results comparable in every way to those obtained from stimulation of the *vagus* in the vertebrates, viz: long after-effects of strong stimulation, and typical escape from weak stimulation.
6. No evidence of acceleration was ever present.

Definitely localized cardiac organs are found in no group of animals generally considered lower than the lamellibranch molluscs. It would therefore seem that, notwithstanding one or two doubtful exceptions, cardiac muscle, wherever found, does not perform its functions entirely independent of inhibitory influences of the central nervous system.

July 27. **The Relation Between the Solution Tension and Physiological Action of the Elements.** By ALBERT P. MATHEWS.

As already pointed out by Loeb and the author, there is a relationship between the valence of an ion and its physiological action. For the motor nerve anions with three charges are somewhat more than three times as powerful stimulants as anions with one charge. There are, however, for other tissues wide divergencies from this rule, particularly for the cathions. For ex-

ample, mercury and copper are enormously more powerful than the bivalent alkaline earth metals; and hydrogen with one charge is more active than other univalent metals. The author suggested that this divergence from the theory probably meant that it was the motion of the valence or charge which ultimately determined its action and that the velocities or orbits differed for the charges attached to different elements. Dr. Stieglitz suggested that the affinity of the atom for its charge might vary and account for these discrepancies. It occurred to the writer that this affinity might be represented by the solution tension of the element. The more easily the element is separated from a solution of its ions, the less is its affinity for the charge it carries as an ion. I have accordingly compared the poisonous action of the metals and negative elements with their relative solution tension as given by Nernst, using *Fundulus* eggs as test objects. The least concentration of the solution of the chlorides of the different metals which would just prevent the formation of an embryo was determined.

The result showed a marked inverse ratio between the solution tension and poisonous action. Potassium with its great solution tension is almost the least poisonous; silver with a very low tension is the most poisonous. The other metals arranged themselves in proper order, except that ferric iron, zinc and cadmium were more poisonous than their position in the list of metals would indicate. This discrepancy is probably due to the fact that the solution tension is but a poor measure of affinity between the charge and the element. The anions arrange themselves in order, those parting with their negative charges most easily being most poisonous.

The correspondence is so close as to indicate clearly that the affinity between the electric charge and the atom is a powerful factor in determining physiological action, and that the less strongly the charge is held, the more powerful is the action of any ion.

Experiments were also tried demonstrating that the action of any cathion is modified by the action of the anion; and that of any anion is modified by the action of the cathion. There is hence an antagonistic action between anions and cathions and the

physiological action of any salt is equal to the sum of the actions of the two ions.

In the toxic and antitoxic action of salts as described by Loeb, both the anion and the cathion are of importance. There is no simple relationship of antitoxic action between monovalent and bivalent cathions.

July 31. **The Cranial Nerves of Squalus acanthias.** By O. S. STRONG.

The object of the work is to confirm, using serial sections, the partial analysis of the cranial nerves into their components accomplished by means of gross dissection by previous investigators, and to extend that analysis further. The components are found to be the same and to have the same general typical arrangement as in other vertebrate types. The trigeminus is purely "general cutaneous." The facialis has four roots: two lateral line roots (R. ophthalmicus superficialis, buccalis, mandibularis externus, etc.) distributed to the canal and ampullary organs of the head, one communis root to the mouth (R. palatinus, mandibularis internus, etc.) and one motor root. The post-auditory roots are the lateral line root, including also a small separate root to one or two canal organs *via* the glossopharyngeus, a series of communis roots and a series of motor roots. The communis roots are distributed into the pre- and post-branchial and visceral branches, innervating the branchial cavities, pharynx and viscera.

July 31. **A Case of Almost Complete Absence of the Left Cerebellar Hemisphere in the Brain of a Child Three Years and Four Months Old.** By O. S. STRONG.

The external appearances have been reported elsewhere (*Journ. Comp. Neur.*, Vol. XI., No. 1). The sections show the following principal abnormalities: absence, usually complete or nearly so, of the right olive and the cerebello-olivary fibers connected with it, of the left restiform body, of the transverse pontine fibers to the left cerebellar hemisphere, of the right *nucleus pontis* and of the left superior peduncle. The left *formatio reticularis* contained more longitudinal fibers than the right. The left lemniscus showed some degeneration and the left *corpus quadrigeminum anterior* was much smaller than the right.

July 29. **Hybrids from Wild Species of Pigeons, Crossed inter se and with Domestic Races.** By C. O. WHITMAN.

The species thus far employed with some success in crossing are the following:

FERAL SPECIES:

1. Oriental turtle (*Turtur orientalis*).
2. European turtle (*Turtur turtur*).
3. Chinese turtle (*Spilopelia*[1] *chinensis*).
4. Surat turtle (*S. tigrina*).
5. Blond ring dove (*Streptopelia*[2] *risoria*).
6. White ring dove (*S. alba*).
7. Oriental ring dove (*S. torquata*).
8. Red ring dove (*S. humilis*).
9. Passenger pigeon (*Ectopistes migratorius*).
10. Mourning dove (*Zenaidura carolinensis*).
11. White-winged pigeon (*Melopelia leucoptera*).
12. Wood pigeon (*Columba palumbus*).
13. Guinea pigeon (*Columba guinea*).

DOMESTIC RACES:

14. Homer (*Columba tabellaria*).
15. Fantail (*Columba laticauda*).
16. Tumbler (*Columba gyrans*).
17. Archangel (*Columba illyrica*).
18. Mondain (*Columba admista*).
19. Chequered rock (*Columba affinis domestica*).
20. Owl-rock hybrid (*C. turbata* × *C. livia*).

The more important hybrids and their parent species were exhibited, and the importance of *known ancestry*, for definite results, was clearly demonstrated.

With a few exceptions, the hybrids were remarkably close intermediates. Reciprocal crosses gave like hybrids.

Several series of fertile hybrids have been obtained. The most remarkable case was a male hybrid between a male chequered rock pigeon (*C. affinis domestica*) and a female oriental

[1] Sundev, *Math. Nat. Av. disp. Tent.*, p. 100, 1872. The species 1–8 are usually included in the genus *Turtur*. The *Streptopelias* and *Spilopelias* are both sufficiently distinct for generic rank, and it is convenient to deal with them as genera.

[2] *Bp.-Comsp. Av.*, II., p. 63, 1854.

turtle dove (*Turtur orientalis*). The offspring of this hybrid, mated with the domestic pigeon, exhibited segregation; but neither in the first nor the second generation were there "dominants" and "recessives" in the Mendelian sense.

No support was found for the so-called "principle of pure germ-cells" in hybrids. Segregation is probably never complete; and in some cases at least the intermediate character seems to be permanent.

August 3. **On the Presence of Specific Coagulins in Animal Tissues.** By Leo Loeb.

It has been known before that extracts of many animal tissues have an accelerating effect upon the coagulation of the blood. It can, moreover, be shown that there exist in animal tissues specific substances which act specifically upon the blood of animals in which they are found, or upon the blood of related species. I have previously found these specific substances in mammals, birds, reptiles and amphibia. Further investigations show that they exist also in invertebrates. The muscle of the lobster acts more strongly upon the blood plasma of the lobster than upon the blood-plasma of the blue crab; the muscle of the blue crab acts more strongly upon the blood-plasma of the blue-crab than upon the blood-plasma of the lobster. Pieces of muscle of other animals than arthropods, which have so far been tried, are without effect upon the blood-plasma of the lobster.

For vertebrate blood there exist non-specific or less specific substances like peptone. Similar substances can also be found in certain animal tissues. The specificity of blood-coagula of vertebrates could not be demonstrated among vertebrates, although they become specific if used with invertebrate blood upon which they do not act at all.

Non-specific or less specific substances exist also in invertebrate tissues, as for instance in the ova of *Arbacia* and other animals. They are able to cause a coagulation of the blood of invertebrates which are not closely related.

The origin of these substances, which are peculiarly useful, will probably have to be explained by a process of auto-immunization. This explanation is suggested by the great similarity be-

tween the specific coagulins and substances derived by artificial immunization.

August 5. **The Retinal Nerve-endings in the Eye of Pecten.** By IDA H. HYDE.

Although the more important methods of staining were tested, none proved so satisfactory as the methylin blue method of Bethe, which was used in a somewhat modified form.

The chief facts regarding the nerve-structure of the eye are as follows: There are two kinds of nerve-tissue. The first is an efferent, or, possibly, a trophic nerve-system, the fibers of which form the side-branch of the optic nerve, and are continuous throughout the peculiar twine-cells to their terminations on the supporting as well as on the nerve-cells in the eye. These fibers penetrate the eye on one side and form a single layer of large unique structures called by the author "twine-cells," as they have the appearance of fibers much coiled in the form of balls of twine. This layer of twine-cells lies between the lens and the retinal disc and on the disc. From these cells fibers extend to other twine-cells, to the tapetum and argentia, as well as to the supporting cells of the retina and as far as the rods.

The fibers are very fine, have a beaded appearance, and are in every respect different from the sensory nerve-fibers, which form the second kind of nerve-tissue.

These sensory nerve-fibers arise as modified nerve-cells, and form the rods which connect with long bipolar cells by means of small dendritic processes. The bipolar cells join the large marginal ganglia by means of nerve-endings in the ganglion. The marginal ganglia form a border of large ganglia cells around the retinal disc, the axones of the ganglia passing in the form of a cup-shaped case over the eye to form the optic nerve. The sensory nerve fibers and cells are surrounded by a hyaline sheath, which is absent in the efferent fibers.

August 5. **The Static Function in Gonionemus.** By LOUIS MURBACH.

The movements of the medusa *Gonionemus* would indicate that it has a definite sense of equilibrium, and some preliminary experiments confirmed this view. To localize this sense experiments

were made to change the center of gravity by weights, etc. These were followed by the removal of different organs, the tentacles, the manubrium and the gonads, but without producing any marked disorientation. It seemed, then, that the otocyst organs to which this function is usually ascribed were thus shown to be active.

At first, solution of the otoliths was attempted with various acids; but it was found that all acids tried, if strong enough to dissolve the otoliths, also killed the animals. Then the vesicles were punctured, putting the otocysts out of function, but in the light of all the experiments there was no definite disorientation, except when the velum was severely mutilated. While this was not a proof that the otocyst organs do not function as the principal organs of equilibrium, the latter observation suggested that equilibration in this medusa is, to a large extent, to be ascribed to "muscular sensation." Further experiments were finally made, cutting away the margin of the bell, including the bases of the tentacles and the otocyst organs. Intermediate portions of the margin were left until healing had taken place; then the remaining portions of the margin were cut away and the animal tested. Although the medusa was more or less imperfect from the operation it was not seriously disoriented but moved in definite directions, including swimming to the surface of a shallow dish of sea water and turning over, also lying with the opening of the bell turned up.

From all the experiments it seems to follow that the otocyst organs serve very little in the equilibrium of the medusæ, and that the muscular sensation is probably the principal factor.

August 7. **Nestling and Juvenile Plumages of Sterna hirundo and S. dougalli.** By Lynds Jones.

The ventral downs of the nestling plumage are wholly white, except the throat, where the tips are dusky-black. All dorsal downs have a dusky-black base, then a tawny area, then a dusky-black area, and many a tawny tip. In the nestling *hirundo* these dusky tips are arranged in mottled pattern, but in *dougalli* in stripes. Both patterns are protective, *dougalli* nesting mostly among grasses, *hirundo* originally on the beach among pebbles and seaweed.

The juvenile plumages of the two species present the same general pattern, but differ in detail. In both the ventral plumage is essentially pure white, *dougalli* showing a faint rosy tint. All dorsal feathers are pure white at the base, then, except the remiges and rectrices, which are nearly like the adult feathers, a pearl-gray area, followed by a dusky area, and all feathers with a tawny tip. The inner tertiaries and their lower coverts, and the lower rows of scapulars, have an added area of tawny and of dusky colors. Thus the tawny and dusky areas of the nestling downs are reproduced in the juvenile feather, with the addition, at the inner half of the juvenile feather, of an area of pearl-gray and a white base. The pearl-gray area corresponds in color to the color of the adult feather.

In *hirundo* these markings produce a barred effect, except on the head, which is white at the base of the bill, gradually darkening to black on the crown and occiput. In *dougalli* the outer tawny and dusky areas are parallel to the border of the feather, in the more strongly marked feathers, for fully a third of the length of the feather, and therefore present a more mottled pattern. Some feathers are even distinctly barred with dusky color about their outer third.

The essential pattern of the two plumages is, therefore, a definite barring of each feather on the dorsal surface, and a lack of any marking on the ventral surface. The greatly modified remiges and rectrices closely resemble the adult remiges and rectrices, thus requiring no transition stage, but the rest of the dorsal plumage undergoes a distinct transition from the nestling to the adult, the intermediate, or juvenile plumage, resembling the nestling plumage for its outer half, at least, and the adult plumage for its basal half.

August 7. **The Internal Factors of Regeneration in Alpheus.** By CHARLES T. BRUES.

Alpheus is a small decapod crustacean in which one of the chelæ is larger than the corresponding one on the other side. When one of the larger chelæ is cut off at the base, it has been shown by Przibram, and also by Wilson, that its stump regenerates a chela of the small type, while the originally small one is

remodeled into a large one. Section of the nerve at the base of the small chela inhibits this reversal, wholly or partially.

With regard to the internal factors which induce this reversal it seems at first sight that the nervous system must be the controlling one. Histological examination shows the following facts: (1) Nerves of the right and left sides are not morphologically different except that they branch differently in the two types of claw. (2) During regeneration or remodeling changes occur in their definitive places, *i. e.*, growth does not occur at one end only. (3) The nervous shock of amputation may produce a weakness of the cut side, and also add to the nutriment of the other side of the ganglion, thus inducing reversal. (4) The nerve evidently carries the stimulus to grow, but may be only passive. (5) The cutting of the nerve in the claw may cause a general disease of that organ, such as might be caused by injuring the musculature, and thus prevent perfect reversal.

It appears, therefore, that we must not attribute to the nervous system as much importance in the remodeling as would seem at first sight necessary when it is found that section of the nerve inhibits reversal.

August 12. **The Development of the Vascular System of Ceratodus.** By Wm. E. Kellicott.

The vascular system of the adult *Ceratodus* shows resemblances to both the elasmobranchs and the amphibia. It was thought that the investigation of its development might throw some light upon the significance of this curious combination. A few of the points of interest which appeared in the course of the study are the following:

The heart develops similarly to that of the frog or *Urodele*, from a pair of folds of the splanchnopleure, the somatopleure forming the pericardium.

The branchial arteries also develop similarly to those of the amphibia. They are four in number and early form continuous passages from the ventral to the dorsal aorta. Later they divide into the afferent and efferent vessels and finally each efferent divides, forming the two efferent branchials characteristic of the adult. The vessels of the hyoid arch are well developed early,

but later disappear. The vessels of the mandibular arch are only slightly indicated.

The veins are arranged symmetrically during the early stages. The arrangement of the cardinal veins is typical. A well-marked subintestinal vessel is present very early; later it disappears in part, and in part becomes connected with the hepatic-portal vein. The vena cava is for a time independent of the cardinal system; later the anterior part of the right posterior cardinal disappears, while the vena cava connects with its posterior section. The pulmonary vein appears very late.

Many of the features of the development of the vascular system are closely similar to those of the Urodela. Some, though not all, of the elasmobranch resemblances appear quite late in the process of development and are preceded by conditions which are typically amphibian. There are, however, typical elasmobranch characters present from the first.

August 12. **The Metamerism of the Nervous System in Arenicola cristata.** By C. P. Lommen.

The arrangement of the nervous system of *Arenicola cristata* is definitely related to the annuli forming its somites. A pair of nerves from the ventral cord proceed dorsally between the longitudinal and circular muscles along each of the grooves that separate the annuli. These nerves give off numerous tiny branches, and do not unite dorsally to form rings. In the setigerous annuli there are two additional nerves which are imbedded in the circular muscles, one on each side of the neuropodium. The posterior one divides into two branches, one passing into the gill and the other into the seta-sac. Anteriorly the number of annuli in the somites is gradually reduced from five to two. From the outside of each connective a series of eight nerves is given off, all of which are presumably homologous to the nerves from the cord. This homology has been shown with certainty only in the case of three. Of the remaining five, one innervates the otocyst, the position and muscular connection of which are suggestive of homology with a seta-sac. Fifteen nerves from the inside of the connectives give off some branches to the body-wall and then bend back into the wall of the pharynx, innervating its ventral

and lateral sides, and passing then into the œsophagus. The dorsal wall of the pharynx is supplied by a plexus of nerve-trunks from the brain and the nearest portions of the connectives. The plexuses on the two sides of the body are seldom alike. From the posterior lobes of the brain several nerves pass to the nuchal organ. In the caudal region, the number of annuli in each somite, and with them the number of nerves, may vary from three in the anterior part to nine or ten in the posterior part.

August 14. **The Organization and Orientation of the Ascidian Egg.** By E. G. Conklin.

During this summer I have studied the development of three species of solitary ascidians, viz. *Styela* (*Cynthia*) *partita*, *Molgula Manhattensis* and *Ciona intestinalis*, with the purpose of finding out how much of organization can be recognized in the unsegmented eggs of these animals.

In the living eggs of all these ascidians, but particularly in the first mentioned, one can recognize the substance of the ectoderm, the endoderm and at least a portion of the mesoderm before the first cleavage occurs.

The spermatozoön enters at the vegetative pole in an area of cytoplasm free from yolk and in *Styela* a dense mass of orange pigment aggregates at this spot and slowly spreads over the vegetative hemisphere. Subsequently it withdraws to one side of this hemisphere, thus forming an orange crescent which lies just below the equator on the posterior side of the egg. The vegetative pole then becomes slate-gray in color and the animal pole a light gray. The study of the subsequent development shows that all the axes of the future animal are now established and that the slate-gray substance forms endoderm, the light gray ectoderm and the orange crescent the muscular system of the tadpole. In *Ciona* and *Molgula* the crescent is present as in *Styela*, but is nearly colorless, while the substance of the ectoderm and of the endoderm may also be recognized in the unsegmented egg.

An incidental result of this work is to prove beyond question than Van Beneden and Julin were right in their orientation of the ascidian egg and that the polar bodies are found at the ectodermal and not at the endodermal pole, as Castle has maintained.

August 14. **Rhythms of Susceptibility and of CO_2 Production in Cleavage.** By E. P. Lyon.

Two years ago the author found that the *Arbacia* egg about fifteen minutes after fertilization is very susceptible to lack of oxygen or to KNC. At other times during the first cleavage it is more resistant. This rhythm of susceptibility and resistance recurs in each succeeding cleavage period.

Further investigation shows that cold acts like lack of oxygen. If the eggs are kept at about 0° C. for a number of hours, those which are placed on ice about fifteen minutes after fertilization are much injured, and may wholly fail to develop, while little harm is done to eggs which have passed the critical stage before being cooled. This rhythm recurs in successive cleavages.

Heating the eggs to 33°–38° C. for a few minutes reveals the opposite rhythm. They are most susceptible at the time of cleavage and are little injured ten or fifteen minutes after fertilization or at corresponding stages in the following cleavages.

The CO_2 output of a mass of eggs is greatest at the time of cleavage. At the time when oxygen is most needed, apparently little CO_2 is produced. This shows that the oxygen, in all probability, is needed for synthetic processes and that the CO_2 is produced by splitting and not by oxidation. The rhythm of CO_2 production can be demonstrated also in the second cleavage.

August 17. **The Voices of Pigeons. I. The Voice of the Ring Dove (Turtur risorius).** By Wallace Craig (demonstrations with the doves).

Nearly five hundred species of wild pigeons are known, and, so far as observation goes, each species has a perfectly distinct and constant set of notes. These voices have had a common origin, and the problem is to discover this and trace the derivation of homologous elements.

The work consists of two parts, a study of the voice in each species, and a comparison of different species and their hybrids. The former may be further divided into: (1) A description of the different notes, the attitudes which accompany each, and their whole significance in the life of the bird; (2) the development of the voice in the young; (3) a history of the seasonal changes in voice and behavior in the adult bird. The first and second of these subdivisions were reported upon in this seminar, and the

main facts presented were as follows : The adult ring dove has only three principal calls, but these have a number of modifications, which, together with many expressive movements, afford a considerable variety of expression. All these modes of behavior seem to be strictly inherited. They develop in the young bird very gradually, and in a definite order which is probably also the order of their development in the race.

August 24. **Some Reactions of Mnemiopsis Leydyi (A. Ag.).** By George William Hunter, Jr.

Mnemiopsis orients itself with reference to gravity, being negatively or positively geotropic under differing conditions. It has two characteristic resting positions, one at the bottom with the aboral pole upward and one at the surface of the water with the oral pole upward.

In strong and moderate intensities of light it may be first negatively and later positively photopathic ; to very weak intensity of light it may be positively photopathic. Some evidences of phototaxis are found under strong light, the aboral end being directed toward the light.

The animal reacts toward a moderately strong constant current (one half to three volts) by turning the aboral pole toward the anode and moving to the cathode. A weaker current may cause orientation without movement toward the cathode.

The action of the "make" and "break" upon muscles and cilia depends upon the position of the electrodes and the strength of the current.

Mnemiopsis is relatively more resistant to decrease than to increase in the temperature of the water. Responses to electrical stimulation under conditions of greater heat than normal show decrease in reaction time up to about 29° C., then rapid increase in reaction time. Responses to electrical stimulation under conditions of decrease from normal temperature show little change in reaction time to about 15° C., then a slow increase in reaction time.

August 24. **The Reaction Time of Gonionemus Murbachii to Electric and Photic Stimuli.** By Robert Mearns Yerkes.

This experimental study of the time relations of the neural processes of *Gonionemus* indicates : (1) A reaction time to elec-

trical stimuli of from .6″ to 2.0″ according to the intensity of the stimulus and the position of the organism; (2) a shorter reaction time when the radial canal regions are stimulated than when the inter-radial regions receive the stimulus; (3) rapid fatigue with repetition of the stimulus; (4) a reaction time to photic stimuli of 1″ to 10″ dependent upon conditions; (5) a much quicker reaction to light when the organism is resting subumbrellar surface uppermost (exposed to the light) than when the exumbrellar surface receives the stimulus; (6) a specialization of organs for the reception of photic stimuli; (7) the existence of highly irritable and conductile tissues (nervous system); (8) that excised organs rapidly lose their irritability; (9) that variability of reaction time for comparative work should be expressed in terms of percentage of the reaction time as well as in absolute terms. This relative variability may be called the variation coefficient.

August 24. **The Establishment of "Association by Contiguity" in Hermit Crabs, Eupagurus longicarpus.** By E. G. SPAULDING.

Crabs are naturally positively *heliotropic*. They were taught to react *against* this by feeding them in a darkened portion of aquarium. Coefficient of daily improvement in eight days = 6.00. The darkening screen was removed each time after feeding. After seven days the crabs go behind the screen, when inserted, although no food is there. This proves the existence (*a*) of association between two different stimuli and (*b*) that when one of these, the screen, is presented, the usual effect of the other is internally reproduced. This may be interpreted as *conscious memory*.

August 24. **The Resin Gnat and Three Parasites**: A Study Made in July and August Under the Direction of Mr. Chas. T. Brues. By L. ECKEL.

This study of *Diplosis resinicola* brought to notice three parasites, among a number of additional facts about this insect, whose larval stage is well known to be passed within the lumps of resin that exude from various species of pine, and which pupates within the resin:

1. *Polygnotus pinicola*, a proctotrupid which has been reported as parasitic upon *Diplosis pini-inopis*. It destroys many of the

larvæ of *Diplosis resinicola* in July, twenty transforming in one *Diplosis* larval skin.

2. Two *Chalcis* flies; one a species of *Syntasis*, described now for the first time as *Syntasis diplosidis*, the second as yet undetermined. They destroy many pupæ of *Diplosis resinicola* in early August.

August 26. **On the Artificial Creation of Mixed Nests of Ants.** By Adele M. Fielde.

Natural mixed nests of ants have been described, but such nests are always of ants belonging to the same subfamily, and not more than two species of ants ever inhabit the same nest.

There are two ways in which an artificial mixed nest may be created; one is by depriving all its residents of the sense of smell by removing the funicles of the antennæ; the other is by accustoming all the resident ants from their earliest hours to the odor of each kind of ant that is to occupy the artificial nest. If a nest not larger than a watch-glass be made, and one or more ants from each selected colony be sequestered in this nest, within twelve hours from the moment of hatching, these ants will each touch all the others with the antennæ and will thus become accustomed to and unafraid of the odor of species unlike their own.

An ant reared in isolation will not affiliate with any whose odor differs from its own. Its criterion of correct ant-odor having been formed within three days after hatching, it continues hostile through life to all ants whose odor disagrees with its standard. But by the process of ant-education herein indicated, any ant may be induced to live peacefully with those of a different genus, or even of another subfamily.

Several artificial nests were shown in which ants of four genèra, or of all the three subfamilies, were living together, and in which young ants of one genus were snuggling the queen of another genus.

Were the ants in the artificial nests set free, their unlike requirements relating to temperature, humidity and food would soon separate them; but it is improbable that these individual ants would ever fight with one another on subsequently meeting, although any of them would fight with ants of other colonies than those in which their early companions originated.

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